FATE OF VIRUSES FOLLOWING SEWAGE SLUDGE APPLICATION TO SOILS

By

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This dissertation is dedicated to my parents,
and to Ambrosina, Adrianne and Amanda.

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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In recent years, land disposal of sewage sludge has been viewed as a viable alternative to other disposal practices. However, there is growing concern over the contamination of groundwater and surface waters with microbial pathogens, particularly viruses, present in digested sludge. The major objective of this study was to assess the potential health risk, from viral pathogens, of sludge application to soils.

Poliovirus type 1 (LSc) was found to be largely associated with digested, conditioned-dewatered, chemical and lime-stabilized, chemical sludge solids. Sludge type was found to affect, however, the degree of association between seeded poliovirus and sludge solids. For example, the degree of association between poliovirus and sludge solids

was significantly greater for aerobically digested sludges (95%) than for activated sludge mixed liquors or anaerobically digested sludges (72% and 60%, respectively). The effectiveness of the glycine method in the recovery of solids-associated viruses was also found to be affected by sludge type. Significantly lower mean poliovirus recovery was found for aerobically digested sludges (15%) than for mixed liquors or anaerobically digested sludges (72% and 60%, respectively).

Poliovirus transport studies involving soil cores treated with virus-seeded sludge were conducted under controlled laboratory and saturated flow conditions. A Red Bay sandy loam displayed a substantially greater retention capacity for poliovirus in anaerobically digested sludge than a sandy soil (i.e., Eustis find sand). The Red Bay sandy loam was shown to completely retain poliovirus following the application of conditioned-dewatered, chemical and lime-stabilized, chemical sludge.

Undisturbed soil cores of Eustis fine sand were treated with several inches of virus-seeded (poliovirus and echovirus type 1-Farouk) sludge during a two-year period. The soil cores were exposed to natural conditions and soil temperature, soil moisture and rainfall were monitored. Both viruses were found to be rapidly inactivated in the sludge during the drying process on top of the soil cores. Monitoring of the/top inch of soil revealed that both viruses were inactivated with time and were undetectable after 35 days. Soil leachates collected after natural rainfall (unsaturated flow conditions) were negative for both viruses.

Indigenous enterovirus were not detected in topsoil and groundwater samples from two sludge disposal sites in Florida.

CHAPTER I
INTRODUCTION

In the United States, the Water Pollution Control Act of 1972 (PL 92-500), as recently amended, requires acceptable methods for the utilization and disposal of wastewater effluents and sludges (Willems 1976). It now appears that land disposal of wastewater effluents and sludges is a viable and attractive alternative to other disposal practices. Land spreading of wastewater effluents and sludges has many advantages, including the addition of plant nutrients, water conservation, improvement of soil physical properties, and increased soil organic matter. However, concern has been raised over the contamination of groundwater and surface waters with nitrates, heavy metals, and microbial pathogens, particularly viruses (Bitton 1975; Bitton 1980b; Burge and Marsh 1978; Gerba et al. 1975).

Viruses are generally associated with wastewater solids (Cliver 1976; Lund 1971) and a significant fraction of these viruses is transferred to sludge as a result of wastewater treatment processes. Sludge treatment processes, such as anaerobic digestion, do not completely inactivate or remove viruses (Bertucci et al. 1977). Therefore, the application of anaerobically digested sludge onto land can lead to groundwater contamination as a result of virus transport through the soil matrix. The movement of sludge-associated viruses is probably limited due to the immobilization of sludge solids

in the top portion of the soil profile (Cliver 1976). However, there are "free" viruses which have not become associated with the sludge solids or which dissociated from these solids as a result of changes in the physico-chemical properties within the soil matrix, and which may move through the soil to contaminate groundwaters. The movement of these individual particles through the soil has been reviewed by Bitton (1975) and Gerba et al. (1975), and is dependent on the type of soil, flow rate, degree of saturation of pores, pH, conductivity, and the presence of soluble organic materials.

A multidisciplinary project designed to study the effect of sludge application on crops, land, animals, and groundwater was undertaken by researchers at the University of Florida, and was funded by the U.S. Environmental Protection Agency (Edds et al. 1980). A virus study was included since virtually little is known on the survival and movement of sludge-bound viruses in soils. The major objective of this study was to assess the potential health risk, from viral pathogens, of sludge application to soils. This objective was achieved by studying the following:

1. Effect of sludge type on poliovirus association with and recovery from sludge solids (Chapter III)
2. Poliovirus transport studies involving soil cores treated with virus-seeded sludge under laboratory conditions (Chapter IV)
3. Retention and inactivation of enteroviruses in soil cores treated with virus-seeded sludge and exposed to the North-Central Florida environment (Chapter V)

4. Monitoring of indigenous enteroviruses at two sludge disposal sites in Florida (Chapter VI)
5. Effect of hydrostatic pressure on the survival of poliovirus seeded in groundwater and seawater (Chapter VII--this research was conducted in order to determine virus survival in the groundwater environment).

This research has allowed the determination of the persistence and possible movement of pathogenic viruses in soils treated with wastewater sludge. The information gained from this study is of value in the ultimate assessment of the potential risk of viral infection to humans associated with land disposal of sludges.

CHAPTER II
LITERATURE REVIEW

Viral Pathogens Found in Raw Wastewater

The pathogens found in raw wastewater fall into one of the following four groups: bacteria, protozoa, helminthic parasites, and viruses. Several reviews have appeared in the literature that address all the pathogens found in sewage (Burge and Marsh 1978; Foster and Engelbrecht 1973; Elliott and Ellis 1977). Herein, the emphasis will be on the viral pathogens present in raw wastewater.

Over 100 types of viruses are found in raw wastewater (Bitton 1980b; Burge and Marsh 1978; Foster and Engelbrecht 1973; Elliott and Ellis 1977). The most important virus groups are the enteroviruses (i.e., polioviruses, coxsackieviruses and echoviruses), reoviruses, adenoviruses, infectious hepatitis agent (viral hepatitis type A--Hall 1977), and viral gastroenteritis agents [variously designated as duoviruses, rotaviruses, reovirus-like agents or Norwalk agent (parvovirus)--Chanock 1976]. These organisms cause such diseases as poliomyelitis, aseptic meningitis, myocarditis, enteritis, jaundice, infectious hepatitis, and gastroenteritis (Burge and Marsh 1978; Foster and Engelbrecht 1973).

Indigenous virus concentrations ranging from 500 to 80,000 plaque-forming units (PFU)/ℓ were measured by Buras (1974) in raw sewage from Haifa, Israel. Dugan et al. (1975) found between 27 and 19,000

PFU/ℓ of virus in raw sewage from the Mililani (Oahu, Hawaii) sewage treatment plant. Mack et al. (1962) found a maximum of 62,800 PFU/ℓ of virus in raw sewage. In Austin, Texas, raw sewage, Moore et al. (1977) reported virus concentrations between 140 and 1,490 PFU/ℓ. Wellings et al. (1974, 1975) found virus concentrations ranging from 54 to ⩾ 161 PFU/ℓ in raw sewage from two locations in Florida. Clearly, a wide range of virus concentrations and types is found in raw wastewater. The virus concentration detected in raw wastewater depends on the geographical location, season of the year (Lund et al. 1969) and virus recovery method used (Buras 1974; Foster and Engelbrecht 1973).

Removal of Viruses by Wastewater Treatment Processes

Wastewater treatment processes (Fair et al. 1968; Zoltek and Melear 1978) vary in their ability to remove pathogenic viruses. Several authors have reviewed the literature on virus removal by wastewater treatment processes (Berg 1973a; Elliott and Ellis 1977; Foster and Engelbrecht 1973; Grabow 1968; Kollins 1966; Malina 1976; Sproul 1976). In this section, the emphasis will be placed on the two treatment processes that generate the most sludge. These processes are primary sedimentation, with or without chemical addition, and activated sludge (i.e., secondary treatment).

Primary Sedimentation

Primary sedimentation is the most common and sometimes the only treatment prior to final disposal of wastewater (Kollins 1966; Grabow 1968). The detention time of wastewater in this treatment

process is usually only some hours (Fair et al. 1968). The capacity of primary sedimentation to remove viruses is at best minimal (Foster and Engelbrecht 1973; Kollins 1966; Grabow 1968; Sproul 1976). Clarke et al. (1961) found only 3% removal of seeded poliovirus type 1 (Mahoney) from raw sewage during a three-hour settling period. Chemical flocculation using alum, ferric chloride or lime followed by sedimentation (i.e., intermediate or chemical treatment), however, has been shown to be very effective in the removal of viruses from raw sewage (Berg 1973a; Lund 1976; Malina 1976; Grabow 1968; Sproul 1976). The removal of viruses by chemical flocculation has been tested in the laboratory using suspending media of varying composition (see review by Berg 1973a).

Several laboratory studies, using suspending media other than raw sewage, have shown that seeded viruses are effectively removed during flocculation. Change et al. (1958) reported 86.3 to 98.7% removal of seeded coxsackievirus A2 after flocculation at pH 6.2 in distilled water-SiO_2-$NaHCO_3$ buffer with 40 to 100 ppm of alum [as $Al_2(SO_4)_3$], respectively. The removal of this virus by alum flocculation conformed to the Freundlich isotherm and, therefore, these investigators concluded that the removal mechanism was adsorption. Approximately 60% of the virus associated with the aluminum flocs was recovered following elution with 0.1 M $NaHCO_3$, at a final pH 8.5 (see Chang et al. 1958). In a similar study involving phosphate precipitation from water, Brunner and Sproul (1970) found 89 to >98% removal of seeded poliovirus type 1 (Sabin) from distilled water-phosphate medium following alum [68 mg/ℓ as $Al_2(SO_4)_3$] flocculation. The removal measured was attributed to the

adsorption of the virus to the aluminum phosphate flocs and, therefore, the removal efficiency increased with a corresponding increase in the quantity of phosphate precipitated. These investigators also found that the removal efficiency of poliovirus was influenced by pH with maximum removal (i.e.., >96%) observed at pH 6.4, and a reduction in removal at pH 5.1 and 7.3. Moreover, only 40% of the polioviruses associated with the aluminum phosphate flocs were recovered when the flocs were dissolved in 0.1 N NaOH, final pH 8.3 to 9.3 (see Brunner and Sproul 1970). These investigators attributed their lack of recovery of the adsorbed viruses to viral inactivation during the precipitation process. However, it is likely that the method used to dissolve the flocs was inefficient in the recovery of the adsorbed viruses. Brunner and Sproul (1970) also studied the removal of poliovirus (93 to >97%) from filtered wastewater effluent during phosphate precipitation with alum. In this medium, virus removal increased with decreasing pH from 6.9 to 5.0. This was attributed to an increase in suspended solids removal with decreasing pH. Bacteriophages have also been effectively removed (93 to >99%) from water following flocculation with alum (Brunner and Sproul 1970; Chang et al. 1958; Chaudhuri and Engelbrecht 1972; York and Drewry 1974).

In addition to alum, ferric chloride has also been tested as a coagulant in the removal of viruses from water. Chang et al. (1958) reported 96.6 and 98.1% removal of seeded coxsackievirus A2 after flocculation at pH 6.2 in distilled water-SiO_2-$NaHCO_3$ buffer with 20 and 40 ppm of ferric chloride (as $FeCl_3$), respectively. Similarly, Sobsey

et al. (1977) studied the flocculation of poliovirus type 1 (LSc) by ferric chloride. These investigators added the virus to a membrane concentrate (i.e., 0.5 M glycine buffer) of turbid estuary water and proceeded to concentrate the virus by flocculation. At a concentration of 0.001 M $FeCl_3$, >99% of the seeded viruses were removed from the supernatant at pH values from 3.5 to 7.5. However, maximum precipitation was observed at pH 3.5. Bacteriophages have also been removed efficiently from water (>99% removals) by flocculation with ferric chloride (Change et al. 1958; York and Drewry 1974). In fact, bacteriophage removals consistently exceeded those of enteroviruses (Chang et al. 1958). Chang et al. (1958) found ferric chloride to be more efficient, on a molar basis, than alum in the removal of coxsackievirus A2 from water by flocculation. Furthermore, these researchers noted that the flocs formed with ferric chloride were more compact and settled more rapidly than those formed with alum. Chang et al. (1958) were unsuccessful in recovering the coxsackieviruses associated with the iron flocs by eluting with 0.1 M $NaHCO_3$, final pH 8.5. It is likely that this solution was inadequate as an eluent for the recovery of the adsorbed viruses, although viral inactivation during the flocculation process cannot be ruled out. In contrast, 74% of the polioviruses associated with the iron flocs were recovered by Sobsey et al. (1977) following elution with fetal calf serum (FCS) at pH 8.0.

Calcium hydroxide (i.e., lime) is another chemical frequently used in flocculation tests for the removal of viruses from water. Sproul (1972) has reviewed the literature on virus removal by water-softening precipitation processes involving lime as the coagulant.

Removals in excess of 99% have been reported for poliovirus type 1 during excess lime-soda ash softening at pH 10.8 to 11.2 (Wentworth et al. 1968). Enteroviruses are actually inactivated under the high pH conditions achieved rather than merely removed by the flocculation process (Sproul 1972). The inactivation of enteroviruses under alkaline pH is believed to be caused by the denaturation of the protein coat and the subsequent disruption of the structural integrity of the virus (Sproul 1972). Lime flocculation followed by sedimentation or/and sand filtration is frequently used as a tertiary (advanced) treatment of secondary wastewater effluents (Malina 1976). This treatment effectively removes suspended solids and phosphates from secondary effluents (Berg et al. 1968). Several studies (field and laboratory) have been undertaken to determine the removal efficiency of viruses from secondary effluents by lime flocculation. Brunner and Sproul (1970) performed laboratory precipitation tests involving the addition of lime [as $Ca(OH)_2$] to filtered wastewater effluent until the pH was raised to between 9.5 and 10.9. Poliovirus type 1 (Sabin) removals ranging from 88 to 94% were achieved. Generally, virus removal increased with a corresponding increase in pH and in the quantity of phosphate precipitated. In a similar laboratory study, Berg et al. (1968) showed that 70 to >99% of poliovirus type 1 (LSc) seeded in secondary wastewater effluent was removed by lime flocculation [with 300 mg/ℓ (pH 10.2) to 500 mg/ℓ (pH 11.0) of $Ca(OH)_2$, respectively] followed by sedimentation. These investigators also observed that virus removal increased as the pH achieved by the addition of lime increased. Additional experimentation confirmed that poliovirus was

inactivated by the high pH produced and that the viral inactivation rate increased as the pH was raised from 10.1 to 11.1 (see Berg et al. 1968). Therefore, these researchers concluded that the removal of poliovirus by this treatment process resulted from a combination of viral inactivation and physical separation of the virus by the flocculation process. The effectiveness of lime flocculation in the removal of viruses from secondary effluents has also been shown in the field. At a wastewater reclamation plant, Grabow et al. (1978) showed that >99.9% of indigenous enteric viruses in activated sludge effluent were removed by lime flocculation at pH 9.6 to 11.2 followed by sedimentation. Coliphages, enterococci and coliform bacteria were also effectively removed by the lime flocculation process (see Grabow et al. 1978).

Chemical flocculation using alum, ferric chloride or lime is frequently combined with sedimentation in the primary treatment of raw sewage (U.S. Environmental Protection Agency 1973). Among the advantages of this treatment process are low capital costs, minimal space requirements and high reliability (Weber et al. 1970). In laboratory-scale experiments, Shuckrow et al. (1971) showed that the flocculation of raw sewage with alum was highly efficient in removing suspended solids, total organic carbon (TOC) and chemical oxygen demand (COD). Similar results were obtained by Weber et al. (1970) when lime or ferric chloride were used as coagulants. Substantial removals of phosphates, nitrates, and organic color are also achieved by this treatment process (Sproul 1976; Weber et al. 1970). As reviewed in the previous paragraphs, numerous studies have shown that viruses are effectively removed from

water and wastewater effluents by flocculation with alum, ferric chloride or lime. Unfortunately, only a few studies have been conducted to determine the effectiveness of chemical flocculation in removing viruses from raw sewage (see reviews by Berg 1973a; Lund 1976; Malina 1976; Sproul 1976). In laboratory-scale pilot plants, Lund and Rønne (1973) studied the fate of indigenous enteric viruses in raw sewage following flocculation with alum [75 to 175 mg/ℓ as $Al_2(SO_4)_3$], ferric chloride (25 to 35 mg/ℓ as $FeCl_3$) or lime (added lime until pH 10.5 was maintained). These investigators noted appreciable removals of viruses in all flocculation experiments. The viruses were found concentrated in the chemical sludges produced. Furthermore, no viral inactivation was observed in the chemical sludges. In another laboratory study, Sattar et al. (1976) showed that 99.995% of poliovirus type 1 (Sabin) seeded in raw sewage was inactivated during flocculation with lime at pH 11.5. No viruses were recovered in the supernatants following flocculation and an average of only 0.005% of the total viral input was recovered in the lime sludges by eluting with 10% FCS in saline, pH 7.2. During storage at 28°C, further viral inactivation was noted in the lime sludges and no viruses could be detected in the sludge samples after 12 hours. The inactivation rate of seeded poliovirus type 1 (Sabin) during lime flocculation of raw sewage was reduced as the pH of the solution was decreased. Sattar and Ramia (1978) found that 92.4 and 97.2% of the total inputs of poliovirus were inactivated (i.e., recovered only 7.6 and 2.8% in the supernatants and sludges combined) during lime flocculation at pH 9.5 and 10.5,

respectively. In these studies (Sattar et al. 1976; Sattar and Ramia 1978), it was pointed out that the method used (i.e., elution with 10% FCS in saline, pH 7.2) to recover viruses from the lime sludges may not have been completely effective. In spite of this possible limitation, the authors concluded that substantial viral inactivation can be achieved during the flocculation of raw sewage with lime at high pH.

The literature presented herein indicates that chemical flocculation, under optimal laboratory conditions, can remove large quantities of viruses from raw sewage and from other water samples. Virus removal efficiency depends on the coagulant, coagulant dose, water type, pH, virus type and flocculation procedure employed. Since it is difficult to ensure optimum floc formation routinely in practice, Grabow (1968) hypothesized that, under field conditions, significant virus removals by flocculation are not likely. As proposed by Berg (1973b), more research is needed before a conclusion can be reached as to the virus removal capacity of chemical flocculation in actual practice.

The effluents from the primary sedimentation units generally still contain demonstrable quantities of enteric viruses (Sattar and Ramia 1978). These primary effluents usually undergo further treatment by, for example, the activated sludge process. Large quantities of sludge (i.e., 2,400 to 5,000 gallons of sludge per million gallons of wastewater treated) are produced during the primary sedimentation process (U.S. Environmental Protection Agency 1974). When chemical

flocculation is combined with primary sedimentation, even larger quantities of sludge (i.e., 5000 to 38,000 gallons of sludge per million gallons of wastewater treated) are produced (U.S. Environmental Protection Agency 1974). These raw primary sludges (in particular, the chemical sludges) contain significant amounts of enteric viruses (Berg and Berman 1980; Lund 1976; Lund and Rønne 1973; Nielsen and Lydholm 1980; Sattar and Ramia 1978; Sproul 1976). In fact, the indigenous enteric viruses are concentrated in the chemical sludges (Lund and Rønne 1973). Sattar and Ramia (1978) readily detected indigenous viruses in all the primary lime sludge samples they tested. The sludge samples were obtained from a wastewater treatment plant in Canada which employed lime flocculation of raw sewage at a pH of approximately 10. Clearly, even under such virucidal conditions of alkaline pH, viruses were recovered from the sludges produced. Thus, all primary sludges should be considered to represent potential health hazards, and should be handled with care during further treatment and final disposal (Brunner and Sproul 1970; Lund 1976; Sattar and Ramia 1978).

Activated Sludge

The activated sludge process (i.e., secondary or biological treatment) is perhaps the most effective wastewater treatment process in the removal of viruses. Several authors have reviewed the literature on virus removal by this treatment process (Berg 1973a; Foster and Engelbrecht 1973; Grabow 1968; Kollins 1966; Malina 1976; Sproul 1976). In laboratory-scale pilot plants, 90% or more of the seeded enteroviruses were removed by the activated sludge process (Clarke *et al.* 1961; Malina *et al.* 1975).

In these studies, the seeded viruses were transferred to the settled sludges (Clarke et al. 1961; Malina et al. 1975). Clarke et al. (1961) recovered only a small fraction of the viruses (i.e., poliovirus type 1--Mahoney or coxsackievirus A9) theoretically associated with the settled sludge. These researchers concluded that the viruses were inactivated when adsorbed to sludge particles. However, their failure to adequately recover the adsorbed viruses can partly be attributed to the poor recovery method used (i.e., used buffer solutions and versene as eluents). In a laboratory-scale activated sludge unit, Malina et al. (1975) effectively recovered poliovirus type 1 (Mahoney) from the settled sludge by using a better eluent, distilled deionized water. Moreover, poliovirus associated with the sludge particles was observed to be inactivated over time. These investigators measured the viral inactivation rate in the settled sludge and found that the rate conformed to the following equation:

$$C_t = C_i e^{-k_2 t} \qquad (2\text{-}1)$$

where C_i = virus associated with sludge initially (PFU/mg of dry sludge solids)

C_t = virus associated with sludge at time t (PFU/mg of dry sludge solids)

t = time (min)

k_2 = rate constant (min^{-1})

The rate constant (i.e., k_2) varied with mixed liquor suspended solids (MLSS) concentration. For example, rate constants of 3.17×10^{-3} and

2.5×10^{-3} min^{-1} were determined for MLSS concentrations of 1,590 and 3,140 mg/ℓ, respectively (see Malina et al. 1975).

As far as indigenous viruses are concerned, much less work has been done on their removal by the activated sludge process. Moore et al. (1977, 1978) and Farrah et al. (1981b) readily recovered indigenous enteroviruses from the mixed liquor suspended solids of activated sludge plants in Texas, Illinois, Montana and Oregon, and Florida, respectively. Since in these three studies most of the viruses detected were directly associated with the solids, it can be hypothesized that a large fraction of these viruses would be removed during subsequent secondary sedimentation of the sludge solids. The effectiveness of the activated sludge process in removing indigenous viruses from wastewater was confirmed by Lund et al. (1969) and Moore et al. (1977) in field studies in Denmark and the United States (Austin, Texas), respectively.

In spite of the substantial virus removal capacity of the activated sludge process, effluents from this treatment method routinely contain demonstrable indigenous viruses (Buras 1974; Dugan et al. 1975; England et al. 1965; Gilbert et al. 1976a, 1976b; Merrell and Ward 1968; Moore et al. 1977; Vaughn et al. 1978; Wellings et al. 1974, 1976a, 1978). Further treatment by chlorination (Dugan et al. 1975; England et al. 1965; Merrell and Ward 1968; Vaughn et al. 1978; Wellings et al. 1974, 1978), or by tertiary processes such as oxidation pond, denitrification followed by sand filtration, or alum flocculation (England et al. 1965; Merrell and Ward 1968; Vaughn et al. 1978; Wellings et al. 1978) often does not eliminate all indigenous viruses from activated sludge effluents. In addition to viruses, secondary

effluents also contain bacterial pathogens (Foster and Engelbrecht 1973), parasites (Hays 1977) and a variety of hazardous chemicals (e.g., nitrates, phosphates, chromium, cadmium, mercury, zinc, copper, polychlorinated biphenyls and phthalates; see Lee 1976). Therefore, secondary effluents should be regarded as potential health hazards in their final disposal.

During the activated sludge process, large quantities of sludge (i.e., 14,000 to 19,000 gallons of sludge per million gallons of wastewater treated) are also produced (U.S. Environmental Protection Agency 1974). The volume of waste activated sludge (i.e., secondary sludge) produced is usually much greater than the volume of sludge generated during primary sedimentation, due to the greater moisture content of the former (U.S. Environmental Protection Agency 1974). In fact, when a wastewater treatment plant is upgraded to activated sludge treatment, the capacity for excess sludge handling (i.e., sludge treatment and disposal) must be significantly increased (U.S. Environmental Protection Agency 1974). Indigenous enteric viruses are routinely found in raw secondary sludges (Berg and Berman 1980; Lund 1976; Lund and Rønne 1973; Nielsen and Lydholm 1980). Therefore, all secondary sludges should be considered to represent potential health hazards, and should be handled with care during further treatment and final disposal.

Removal of Viruses by Sludge Treatment Processes

Viruses in Raw Sludges

As reviewed above, raw sludges produced during the primary and secondary treatment of wastewater contain substantial quantities of

enteric viruses. The indigenous virus titer of raw sludge has been measured by several investigators and expressed in different units. In raw primary sludge, indigenous enteric virus concentrations of 2.4 to 15 PFU/ml, $\leq 6.9 \times 10^2$ PFU/ml, 6.9 to 215 PFU/g dry wt. of total suspended solids (TSS), 7.9×10^2 to 4.3×10^3 PFU/g dry wt. TSS and 10 to 1,000 50% tissue culture infective dose ($TCID_{50}$)/ml were measured by Cliver (1975), Nath and Johnson (1980), Turk et al. (1980), Moore et al. (1978) and Lund (1976), respectively. In mixtures of primary (1/3) and secondary (2/3) raw sludge from the City of Los Angeles Hyperion treatment plant, Berg and Berman (1980) found indigenous enteric virus concentrations ranging from 3.8 to 116 PFU/ml. Nielsen and Lydholm (1980) detected 0.1 to 9.0 $TCID_{50}$/mg TSS of indigenous enteric viruses in raw sludge (primary and secondary) from three Danish wastewater treatment plants. Secondary sludges have been shown to contain 10- to 100-fold less virus than primary sludges (Lund 1976; Lund and Rønne 1973). Clearly, a wide range of virus concentrations is found in raw sludges. The indigenous virus concentration detected in raw sludge depends on the sludge type, geographical location, season of the year (Berg and Berman 1980) and virus recovery method used (Nath and Johnson 1980). Moreover, a large fraction of the indigenous viruses in raw sludges have been shown to be associated with the sludge solids (Cliver 1975; Lund 1976; Lund and Rønne 1973; Nath and Johnson 1980). Indigenous enteric viruses are strongly associated with fecal solids in raw wastewater (Bitton 1980a; Cliver 1975; Cliver 1976; Wellings et al. 1976a) and tend to remain associated with solids during

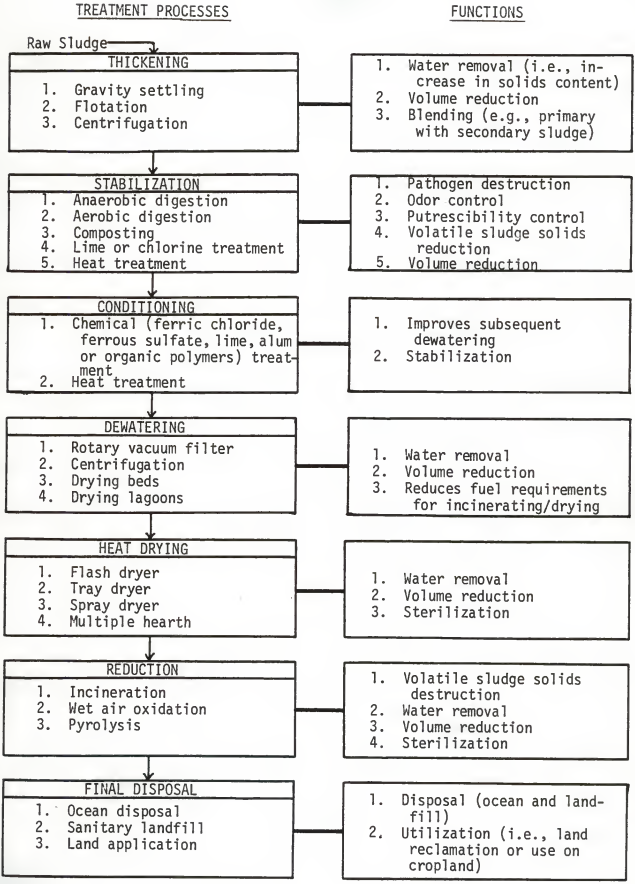
wastewater treatment processes (Cliver 1976). Furthermore, indigenous viruses are believed to be mostly embedded within the sludge solids rather than merely surface adsorbed (Wellings et al. 1976a). This association between indigenous viruses and sludge solids has significant implications on viral survival during subsequent sludge treatment.

Sludge Treatment Processes

A variety of sludge treatment processes is routinely used in treatment plants and they are shown in Figure 2-1. From Figure 2-1,it can also be seen that several often overlapping functions are achieved by each sludge treatment process. The order of the sludge treatment processes shown in Figure 2-1 is as most often used in treatment plants (Fair et al. 1968; Malina 1976; U.S. Environmental Protection Agency 1974). However, it should be pointed out that not all sludge treatment processes shown in Figure 2-1 are employed at every treatment plant. Moreover, the order of the sludge treatment processes may be varied from that shown in Figure 2-1 depending on the existing conditions. Characteristics of the sludge treatment processes have been reviewed by several authors (Dick 1978; Fair et al. 1968; Malina 1976; U.S. Environmental Protection Agency 1974, 1978a, 1978b; Yates 1977). Some work has been done on the removal of viruses (i.e., by physical separation and/or inactivation of viral particles) from raw sludges (i.e., primary, chemical, and/or secondary sludges) by sludge treatment processes and is reviewed below.

FIGURE 2-1. Outline of sludge treatment processes and their functions

Adapted from Fair et al. (1968), Malina (1976) and United States Environmental Protection Agency (1974, 1978a, 1978b).


TREATMENT PROCESSES
FUNCTIONS
Raw Sludge
THICKENING
1. Gravity settling
2. Flotation
3. Centrifugation
1. Water removal (i.e., increase in solids content)
2. Volume reduction
3. Blending (e.g., primary with secondary sludge)
STABILIZATION
1. Anaerobic digestion
2. Aerobic digestion
3. Composting
4. Lime or chlorine treatment
5. Heat treatment
1. Pathogen destruction
2. Odor control
3. Putrescibility control
4. Volatile sludge solids reduction
5. Volume reduction
CONDITIONING
1. Chemical (ferric chloride, ferrous sulfate, lime, alum or organic polymers) treatment
2. Heat treatment
1. Improves subsequent dewatering
2. Stabilization
DEWATERING
1. Rotary vacuum filter
2. Centrifugation
3. Drying beds
4. Drying lagoons
1. Water removal
2. Volume reduction
3. Reduces fuel requirements for incinerating/drying
HEAT DRYING
1. Flash dryer
2. Tray dryer
3. Spray dryer
4. Multiple hearth
1. Water removal
2. Volume reduction
3. Sterilization
REDUCTION
1. Incineration
2. Wet air oxidation
3. Pyrolysis
1. Volatile sludge solids destruction
2. Water removal
3. Volume reduction
4. Sterilization
FINAL DISPOSAL
1. Ocean disposal
2. Sanitary landfill
3. Land application
1. Disposal (ocean and landfill)
2. Utilization (i.e., land reclamation or use on cropland)

Stabilization-digestion. Raw sludges are frequently stabilized using anaerobic (Fair et al. 1968; U.S. Environmental Protection Agency 1974, 1978a) or aerobic (U.S. Environmental Protection Agency 1974, 1978a; Yates 1977) digestion. These digestion processes reduce the odor, putrescibility potential and pathogen content of raw sludges (U.S. Environmental Protection Agency 1974, 1978b) and thereby achieve sludge stabilization (see Figure 2-1).

There are several different design-types of anaerobic sludge digestion and they employ either no heating, heating at mesophilic (30-35°C) temperatures, or heating at thermophilic (approximately 50°C) temperatures (U.S. Environmental Protection Agency 1974). In general, the rate of sludge stabilization increases as the anaerobic digestion temperature is increased. Consequently, the sludge digestion time is usually reduced as the anaerobic digestion temperature is increased (U.S. Environmental Protection Agency 1974).

Several authors have reviewed the literature on viral inactivation during anaerobic digestion of raw sludges (Berg 1973a; Bitton 1978; Cliver 1976; Foster and Engelbrecht 1973; Moore et al. 1977, 1978). In laboratory and field studies, enteroviruses have been shown to be inactivated during anaerobic digestion (Bertucci et al. 1977; Berg and Berman 1980; Cliver 1975; Eisenhardt et al. 1977; Moore et al. 1977, 1978; Nielsen and Lydholm 1980; Palfi 1972; Sattar and Westwood 1979). During anaerobic digestion (35°C) of sludge in laboratory-scale units, Eisenhardt et al. (1977) measured the inactivation rate of seeded

coxsackievirus B3 at 2 $\log_{10}$ units per 24 hours. In similar laboratory anaerobic digesters, Bertucci et al. (1977) observed the inactivation rates of enteroviruses [i.e., poliovirus 1 (Sabin), coxsackievirus A9 (Griggs), coxsackievirus B4 (JVB) and echovirus 11 (Gregory)] seeded in sludge to follow a first-order reaction pattern and to significantly differ for the four viruses tested.

Ward and his collaborators have done a great deal of work on the inactivation of enteric viruses seeded in raw and anaerobically digested sludges. Poliovirus type 1 (CHAT) and poliovirus type 1 (Mahoney) seeded in anaerobically digested sludge (6% sludge solids, pH 8.0) became largely associated (67 and 65% of the total virus added, respectively) with the sludge solids and both were inactivated at rates of approximately 1 $\log_{10}$ units/5 days at 4°C, >2 $\log_{10}$ units/3 days at 20°C, and >1 $\log_{10}$ units/day at 28°C (Ward and Ashley 1976). Anaerobically digested sludge displayed no detectable virucidal activity against enteroviruses when adjusted to pH values between 4.5 and 7.5 (Ward and Ashley 1977a). Similarly, there was no appreciable inactivation of seeded poliovirus type 1 in 5 days at 20°C in raw sludge maintained at its naturally low pH of 6.0 (Ward and Ashley 1976). However, the virucidal activity against poliovirus in raw sludge significantly increased as the pH of the sludge was raised above 7.5 (Ward and Ashley 1977a). Thus, Ward and Ashley (1977a) demonstrated that the uncharged form of ammonia, which exists mostly at pH values above 8, was the causative agent in the irreversible inactivation of enteroviruses in anaerobically digested sludge and in

raw sludge adjusted to pH values above 7.5. Moreover, this agent was shown to be present mainly in the sludge supernatant (i.e., produced by centrifuging anaerobically digested sludge at 18,000 x g for 20 minutes) rather than in the sludge solids (Fenters et al. 1979; Ward and Ashley 1976). Microbial activity in anaerobically digested sludge supernatant was found not to affect the inactivation rates of seeded poliovirus 1 (Sabin), echovirus 6 and coxsackievirus B4 (Fenters et al. 1979). The mechanism of inactivation of poliovirus type 1 (CHAT) in anaerobically digested sludge was found to be cleavage of the two largest viral coat proteins (i.e., breakdown of VP-1 and VP-2) followed by nicking of the encapsulated RNA (Ward and Ashley 1976). At the thermophilic temperature of 43°C, the inactivation rate of seeded poliovirus type 1 (CHAT) was significantly lower in raw and anaerobically digested sludge than in phosphate-buffered saline (PBS) (Ward et al. 1976). Ward et al. (1976) proposed that poliovirus was protected from heat inactivation by a component found in the sludge solids. In the case of anaerobically digested sludge, however, this protective effect was always less than that observed for raw sludge and was largely reversed at higher temperatures (i.e., 47 and 51°C) due to the presence of the virucidal agent, uncharged ammonia (Ward et al. 1976). Thus, at the higher temperatures, the inactivation rates of poliovirus in anaerobically digested sludge were similar to those in PBS (Ward et al. 1976). Ward et al. (1976) also demonstrated that enteroviruses in anaerobically digested sludge were irreversibly inactivated (RNA molecule hydrolyzed) during heating at temperatures of approximately

50°C. At the low natural pH of the raw sludge, the virucidal agent was not present (Ward and Ashley 1976, 1977a). Therefore, only the protective effect attributed to the sludge solids was observed for this sludge type at all temperatures tested (i.e., 43 to 51°C) (Ward et al. 1976). Ionic detergents were later identified as the components in raw and anaerobically digested sludge solids which protected poliovirus type 1 (CHAT) and other enteroviruses from heat inactivation (Ward and Ashley 1977c, 1978a, 1979). In contrast, these ionic detergents (cationic more active than anionic) were shown to be responsible for reducing the heat required to inactivate reovirus type 3 (Dearing) in raw and anaerobically digested sludge (Ward and Ashley 1977c, 1978a). Ward and Ashley (1979) demonstrated that two ionic organic detergents, sodium dodecyl sulfate and dodecyltrimethylammonium chloride in buffer solutions, were potent virucidal agents for reovirus, but that their virucidal effects were strongly pH dependent. The virucidal activity against reovirus displayed by these ionic detergents was greater in alkaline than in acid conditions (Ward and Ashley 1977c). Further research (Ward and Ashley 1979) revealed that the inactivation pattern of reovirus as a function of pH at 45°C in anaerobically digested sludge was qualitatively similar to that found in buffer solutions containing ionic detergents.

The literature reviewed in the paragraph above indicates that the inactivation of enteric viruses in anaerobically digested sludge is a complex phenomenon. It is clear, however, that the anaerobic digestion process raises the pH of raw sludge and thereby produces in the

digested sludge the uncharged form of ammonia which is virucidal for enteroviruses. The presence of this virucide coupled with thermophilic digestion temperatures (i.e., approximately 50°C) results in the rapid inactivation of enteroviruses in anaerobically digested sludge. Furthermore, the protective effect towards enteroviruses attributed to ionic detergents in the sludge solids and observed at low pH in raw sludge is largely overcome by the virucidal ammonia in anaerobically digested sludge (Ward and Ashley 1977c) particularly at thermophilic temperatures. Reoviruses are also inactivated in anaerobically digested sludge at thermophilic temperatures and at the naturally high pH values attained by the digestion process (Ward and Ashley 1979).

As presented above, the inactivation of viruses during anaerobic digestion has been studied in the laboratory using sludge artificially contaminated with virus. The validity of such research has been questioned because it is believed that, unlike indigenous viruses, the seeded viruses become mostly adsorbed to the surface of sludge solids (Moore et al. 1977; Nielsen and Lydholm 1980). Due to their strong association with fecal solids in raw wastewater (Bitton 1980a; Cliver 1975, 1976; Wellings et al. 1976a), indigenous viruses are believed to end up, during wastewater treatment, mostly embedded within sludge solids rather than merely surface adsorbed (Wellings et al. 1976a). Consequently, Moore et al. (1977) proposed that indigenous viruses in sludge are less susceptible than seeded viruses to the environmental stresses (e.g., chemical and heat inactivation) encountered during sludge treatment by virtue of the former's more insulated environment. Evidence to

support this hypothesis has been obtained from studies involving the anaerobic digestion of sludge. At the East Pearl treatment plant in Boulder, Colorado, Moore et al. (1978) found total reductions of indigenous enteroviruses in primary raw sludge of only 2 $\log_{10}$ units (i.e., 99%) during 100 days of anaerobic digestion (40 days at 37°C in digester no. 1 followed by 60 days in unheated digester no. 2). Significantly higher inactivation rates were measured for seeded enteroviruses by Bertucci et al. (1977) and Eisenhardt et al. (1977) during anaerobic digestion of sludge. For example, Eisenhardt et al. (1977) found the inactivation rate of seeded coxsackievirus B3 to be 2 $\log_{10}$ units per 24 hours. The inactivation rates of seeded enteroviruses were drastically reduced when the viruses were incorporated into the sludge during sludge production rather than simply mixed with the final sludge sample. Moore et al. (1977) reported an inactivation rate of approximately 2 $\log_{10}$ units per 15 days for poliovirus naturally incorporated (and assumed embedded) into wasted sludge during activated sludge treatment in a continuous flow, bench-scale unit and then subjected to anaerobic digestion at 30°C. In fact, these investigators detected poliovirus in the sludge undergoing anaerobic digestion even after 30 days. Clearly, enteroviruses embedded within sludge solids are afforded some protection from virucidal chemical agents (e.g., uncharged ammonia in the liquid fraction of anaerobically digested sludge--see Ward and Ashley 1977a) and/or physical stresses (e.g., heat) encountered during the anaerobic digestion of sludge, as well as during other sludge treatment processes.

Although the anaerobic digestion process appears capable of removing considerable quantities of viruses from sludge, a fraction of the viruses initially present will, nevertheless, survive this digestion process [see reviews by Berg (1973a), Bitton (1978), Cliver (1976), Foster and Engelbrecht (1973), and Moore et al. (1978)]. Indigenous viruses have been routinely detected in anaerobically digested sludge (Berg and Berman 1980; Cliver 1975; Farrah et al. 1981a; Moore et al. 1978; Nielsen and Lydholm 1980; Palfi 1972; Sattar and Westwood 1979; Sagik et al. 1980; Turk et al. 1980; Wellings et al. 1976a). Moreover, the indigenous virus titer of this sludge type has been measured by several investigators and expressed in different units. Anaerobically digested sludge sampled at various locations throughout the United States displayed indigenous enteric virus concentrations of 0 to 8 PFU/ml, <0.014 to 4.1 PFU/ml, 7 to 40 PFU/g dry wt. TSS, 1.1 to 17 PFU/g dry wt. TSS, 0.2 to 17.0 PFU/g dry wt. TSS and 2 to 7 $TCID_{50}$/g dry wt. TSS as measured by Cliver (1975), Berg and Berman (1980), Moore et al. (1978), Sagik et al. (1980), Turk et al. (1980) and Farrah et al. (1981a), respectively. Nielsen and Lydholm (1980) found 0 to 600 $TCID_{50}$/g dry wt. TSS of indigenous enteric viruses in anaerobically digested sludge from three Danish wastewater treatment plants. Evidently, the indigenous virus concentration detected in anaerobically digested sludge depends on the virus concentration in the raw sludge, and thereby, on the geographical location (Sagik et al. 1980) and on the season of the year (Berg and Berman 1980; Moore et al. 1978). The indigenous virus titer found in

anaerobically digested sludge also depends on the virus removal efficiency of the anaerobic digestion procedure employed. For example, Berg and Berman (1980) reported that, at the City of Los Angeles Hyperion treatment plant, thermophilic anaerobic digestion (20 days at approximately 49°C) was superior to mesophilic anaerobic digestion (20 days at approximately 35°C) in the removal of indigenous viruses from raw sludge. In the laboratory, Ward et al. (1976) confirmed that the inactivation of seeded poliovirus type 1 (CHAT) in anaerobically digested sludge was accelerated under thermophilic temperatures.

Although little is known about the viral-inactivating capacity of the aerobic digestion process, several investigators have reported that, as in the case of anaerobic digestion, not all indigenous enteroviruses are eliminated from sludge during aerobic digestion (Farrah et al. 1981a, 1981b; Hurst et al. 1978). Farrah et al. (1981a, 1981b) measured indigenous enterovirus titers ranging from 1.7 to 260 $TCID_{50}/g$ dry wt. TSS in aerobically digested sludge from three wastewater treatment plants in Florida. In sludges from two wastewater treatment plants in Pensacola, Florida, Farrah et al. (1981a) showed that aerobically digested sludge contained larger indigenous viral titers than anaerobically digested sludge.

Clearly, both anaerobically and aerobically digested sludges can contain substantial quantities of enteric viruses. Therefore, all digested sludges should be considered to represent potential health hazards, and should be handled with care during further treatment and final disposal (Palfi 1972).

Stabilization-composting. Composting is a biological, aerobic, thermophilic (approximately 60°C) process frequently used to stabilize (see Figure 2-1) raw sludges (U.S. Environmental Protection Agency 1974, 1978b). As such, this process reduces the odor, putrescibility potential and pathogen content of raw sludges (U.S. Environmental Protection Agency 1974, 1978b). Bitton (1980b) described in detail the sludge composting process and reviewed the literature on pathogen destruction during this sludge treatment procedure. Pathogenic parasites and bacteria as well as bacteriophage f2 have been shown to be inactivated during sludge composting (Bitton 1980b). Ward and Ashley (1978b) demonstrated that seeded poliovirus type 1 (CHAT) was heat-inactivated (43°C) at a significantly greater rate in composted sludge than in dewatered raw sludge held at the same sludge solids content of 40%. In the case of seeded reovirus, the reverse was observed (i.e., greater inactivation rate in dewatered raw sludge; see Ward and Ashley 1978b). These viral inactivation patterns were attributed to the effects of sludge solids-associated, ionic detergents (Ward and Ashley 1978b). These detergents were previously shown to influence differently the heat inactivation rate of enteroviruses and reoviruses in sludge. Whereas enteroviruses in sludge were protected from heat inactivation by ionic detergents, reoviruses were inactivated at an accelerated rate (Ward and Ashley 1977c, 1978a, 1979). During composting, however, the ionic detergents in raw sludge were shown to be substantially degraded (Ward and Ashley 1978b). Thus, viral inactivation rates were markedly different in composted sludge as compared to raw sludge and

for enteroviruses versus reoviruses (Ward and Ashley 1978b). Although most enteroviruses are rapidly inactivated during sludge composting, reoviruses apparently are capable of surviving this sludge treatment process (Ward and Ashley 1978b). Clearly, sludge composting does not yield a virus-free product, and therefore, all composted sludges should be handled with care during further treatment and final disposal.

Stabilization-lime treatment. Lime treatment at pH 11.0 to 11.5 is another practice frequently employed to stabilize (see Figure 2-1) raw sludges (Farrell et al. 1974; U.S. Environmental Protection Agency 1974, 1978a). During periods when digesters are out of service or when sludge quantities exceed digester design capacity, lime treatment is an effective alternate method of sludge stabilization (Farrell et al. 1974; U.S. Environmental Protection Agency 1978a). Due to the large quantities of chemical sludges (e.g., alum and iron) usually produced, lime treatment is particularly suited for the stabilization of these sludge types (Farrell et al. 1974). At relatively low costs, lime stabilization reduces the odor, putrescibility potential and pathogen content of raw sludges (Farrell et al. 1974; U.S. Environmental Protection Agency 1974, 1978a, 1978b). However, the effectiveness of this sludge stabilization procedure is apparently dependent upon the pH achieved and maintained. Farrell et al. (1974) demonstrated adequate stabilization of chemical sludges during lime treatment at pH 11.5 for 30 minutes (pH was maintained above 11 for 24 hours). Further research has indicated, however, that the pH must be maintained above 12 for 30 minutes (pH remaining above 11 for at least 14 days) during liming in order to ensure effective sludge stabilization (U.S. Environmental Protection Agency 1974,

1978a). The lime dosage required to exceed, for example, pH 12 for 30 minutes has been found to be affected by the sludge type, chemical composition of the sludge and percent sludge solids (Farrell et al. 1974; U.S. Environmental Protection Agency 1978a). In addition to achieving stabilization, lime treatment also conditions the sludge (see Figure 2-1) such that subsequent sludge dewatering is improved (Farrell et al. 1974; U.S. Environmental Protection Agency 1974).

Most bacterial pathogens in raw sludge have been shown to be destroyed during lime stabilization (Farrell et al. 1974; U.S. Environmental Protection Agency 1974, 1978a). Fecal streptococci, however, remain viable during liming (U.S. Environmental Protection Agency 1978a). Moreover, regrowth of bacterial organisms can occur if the pH of the lime-stabilized sludge is allowed to drop rapidly below 11 (Farrell et al. 1974; U.S. Environmental Protection Agency 1974). Under ideal conditions, lime treatment is superior to anaerobic digestion in the inactivation of bacterial pathogens in raw sludge (U.S. Environmental Protection Agency 1978a). As a result, the bacterial pathogen concentrations in lime-stabilized sludges are 10 to 1,000 times lower than in anaerobically digested sludges (U.S. Environmental Protection Agency 1978a).

As far as enteric viruses are concerned, no research has been conducted on their fate during lime stabilization of raw sludge (Farrell et al. 1974). However, since seeded poliovirus type 1 has been shown to be inactivated during the flocculation of raw sewage with lime at pH 11.5 and to be undetectable (i.e., apparently fully

inactivated) after 12 hours in the lime sludge produced (Sattar et al. 1976), it can be hypothesized that substantial quantities of enteroviruses are probably inactivated during the lime stabilization of raw sludges (i.e., primary, chemical and secondary sludges). This hypothesis has yet to be confirmed experimentally. Until new information becomes available, lime-stabilized sludges should be regarded as potentially containing pathogenic enteric viruses, and, therefore, should be handled with care during further treatment and final disposal.

Stabilization-heat treatment. Heat treatment is yet another process which has been used to stabilize (see Figure 2-1) raw sludges (U.S. Environmental Protection Agency 1974, 1978b). Two types of heat treatment have been used for sludge stabilization, and they are pasteurization at approximately 70°C and low-pressure (180 to 210 psi) oxidation at approximately 200°C (U.S. Environmental Protection Agency 1974). Pasteurization at 70°C for 30 to 60 minutes destroys most pathogens in raw sludge including enteric viruses (U.S. Environmental Protection Agency 1974). Due to the extremely high temperatures (i.e., 200°C) employed during low-pressure oxidation, all pathogens, including enteric viruses, in raw sludge are undoubtedly destroyed (U.S. Environmental Protection Agency 1974, 1978b). Under the most ideal conditions, however, low-pressure oxidation has displayed a poor capacity to reduce the odor and the putrescibility potential of raw sludges (U.S. Environmental Protection Agency 1978b). As shown in Figure 2-1 and described below, heat treatment by the low-pressure oxidation process also conditions the sludge such that subsequent sludge dewatering is

improved (U.S. Environmental Protection Agency 1974, 1978a). It should be pointed out that the heat treatment of sludge is an energy-intensive process. Consequently, in actual practice, the applicability of this treatment procedure is limited due to high energy costs.

Conditioning-chemical treatment. As shown in Figure 2-1, stabilized sludge is frequently conditioned with organic polymers or inorganic chemicals (i.e., ferric chloride, ferrous sulfate, lime or alum) in order to facilitate water removal by subsequent sludge dewatering processes (U.S. Environmental Protection Agency 1974, 1978a). These flocculants provide charge neutralization and thereby aggregate the sludge particles such that a porous, free-draining cake structure is produced (U.S. Environmental Protection Agency 1974, 1978a). Consequently, chemical conditioning improves sludge dewaterability and sludge solids capture during dewatering procedures (U.S. Environmental Protection Agency 1974).

Although never tested, it can be hypothesized that indigenous enteric viruses in sludge are probably associated with the sludge-particle aggregates produced during the chemical conditioning of sludge. Thus, enteric viruses can be expected to be concentrated in the conditioned-dewatered sludge. Except in the case of lime treatment, no significant viral inactivation is likely to result from chemical conditioning.

Conditioning of sludge with lime is routinely undertaken in conjunction with ferric chloride (U.S. Environmental Protection Agency 1974). As shown in Figure 2-1 and described above, lime treatment

also provides stabilization of the sludge (U.S. Environmental Protection Agency 1974, 1978a, 1978b). As such, lime conditioning of sludge reduces odors and the pathogen, including viral, content of sludge (U.S. Environmental Protection Agency 1974, 1978b; also see pages 30 to 32 in this chapter). Until more information becomes available, however, lime-conditioned sludges should not be regarded as virus free.

Conditioning-heat treatment. Heat treatment at temperatures of 300 to 500°F (i.e., 150 to 260°C) and pressures of 150 to 400 psi for periods of 15 to 40 minutes is another conditioning process which facilitates sludge dewatering (see Figure 2-1) (U.S. Environmental Protection Agency 1974, 1978a). Such heat treatment solubilizes and hydrolyzes the smaller and more highly hydrated sludge particles which are then removed from the bulk sludge sample and end up in the cooking liquor (U.S. Environmental Protection Agency 1974). Consequently, heat-conditioned sludge displays a reduced affinity for water and an improved dewatering capacity (U.S. Environmental Protection Agency 1974, 1978a). As shown in Figure 2-1 and described above, heat treatment also leads to the stabilization of sludge (U.S. Environmental Protection Agency 1974, 1978b). Due to the high temperatures employed, all pathogens, including enteric viruses, in sludge are destroyed during heat conditioning (U.S. Environmental Protection Agency 1974, 1978b). Unfortunately, high energy costs often make heat conditioning of sludge impractical.

Dewatering-drying beds. In the United States and Europe, sandbed drying (see Figure 2-1) is the most widely used method for sludge dewatering (U.S. Environmental Protection Agency 1974). Drying

beds consist of 6 to 9 inches (ca. 15 to 23 cm) of sand underlaid with approximately 12 inches (ca. 31 cm) of graded gravel or stone (U.S. Environmental Protection Agency 1974). Criteria for the design of drying beds can be found in the literature (U.S. Environmental Protection Agency 1974, 1978a). In drying beds, water removal from sludge is accomplished first by drainage (filtrate is collected by underdrain system and is returned to the plant for further treatment) and then followed by evaporation (U.S. Environmental Protection Agency 1974). The effectiveness of sludge dewatering in drying beds is influenced by weather conditions (e.g., precipitation, solar radiation, air temperature, and relative humidity), sludge characteristics (e.g., primary sludge dries faster than secondary sludge, digested sludge dries faster than raw sludge, and digested sludge dries faster than lime-stabilized sludge) and prior use of sludge conditioning (e.g., proper chemical conditioning can reduce sludge dewatering time by 50% or more) (U.S. Environmental Protection Agency 1974, 1978a). Sludge solids contents ranging from 45% for well-digested sludge to 90% for chemically conditioned sludge can be achieved on drying beds (U.S. Environmental Protection Agency 1974). As with other dewatering processes, sandbed drying reduces the volume of sludge to be further treated and disposed of (U.S. Environmental Protection Agency 1974). Such dewatering, therefore, reduces the fuel requirements of, for example, sludge incineration (U.S. Environmental Protection Agency 1974).

During the air drying of sludge, it has also been demonstrated that enteric viruses are inactivated. Working with raw sludge (pH 6 or

less; lacking virucidal ammonia), Ward and Ashley (1977b) found a gradual reduction in the titer of seeded poliovirus type 1 (CHAT) during the air drying of sludge at 21°C from 5% to 65% sludge solids content. However, when the sludge was allowed to dry to a solids content of 83% or greater, these investigators observed a dramatic decrease in poliovirus titer of greater than three orders of magnitude in 4 days (similar results were also obtained using seeded coxsackievirus B1 and reovirus 3). Ward and Ashley (1977b) went on to demonstrate that viral RNA is released during the air drying of sludge and this results in irreversible viral inactivation. Moreover, the evaporation process itself, and not some virucidal agent (note that the raw sludge used lacked the virucidal form of ammonia), was found responsible for poliovirus inactivation during the air drying of sludge at 21°C (Ward and Ashley 1977b). In fact, the inactivation rate of poliovirus incorporated into dewatered raw sludge was significantly lower than the inactivation rate of poliovirus seeded in raw sludge and allowed to air dry (Ward and Ashley 1977b). During heat treatment at 47°C or 51°C, the inactivation rate of poliovirus type 1 (CHAT) incorporated into dewatered raw sludge significantly declined as the sludge solids content was increased from 5% to 80% (Ward and Ashley 1978b). Sludge solids content (or sludge moisture content) itself was shown to have an insignificant effect on the rate of poliovirus inactivation by heat (Ward and Ashley 1978b). Sludge solids-associated ionic detergents, however, were found to protect poliovirus in sludge from heat inactivation and to be concentrated during sludge dewatering

(Ward and Ashley 1978b). Hence, the greater protection from heat inactivation afforded to poliovirus as the sludge solids content was increased (Ward and Ashley 1978b). The ionic detergents in raw sludge were shown to be substantially degraded during the composting process and as a result, seeded poliovirus was heat-inactivated (39°C or 43°C) at a greater rate in composted sludge than in raw sludge held at the same sludge solids content (Ward and Ashley 1978b). Other enteroviruses (e.g., poliovirus 2, coxsackievirus A13 and coxsackievirus B1) have also been shown to be protected from heat inactivation in dewatered raw sludge (Ward and Ashley 1978b).

From the research presented above, it can be concluded that substantial viral inactivation would occur, during the air drying of raw sludge at 21°C, only when sludge solids contents above 80% are achieved (Ward and Ashley 1977b). In actual practice, however, such sludge solids contents are rarely attained for raw sludge during sandbed drying (U.S. Environmental Protection Agency 1974). Furthermore, raw sludge is not routinely subjected to air drying because of the odors, insect pests, unsatisfactory drying rate and other problems associated with this practice (U.S. Environmental Protection Agency 1974). Thus, sandbed drying is normally restricted to well-digested sludge (U.S. Environmental Protection Agency 1974). It can be hypothesized that enteroviruses are probably more rapidly inactivated in anaerobically digested sludge than in raw sludge when subjected to air drying. This is because anaerobically digested sludge usually contains virucidal ammonia (Ward and Ashley 1976, 1977a). This agent has been shown to

reverse the protective effect towards enteroviruses attributed to ionic detergents associated with sludge solids (Ward and Ashley 1977c, 1978a, 1979; Ward et al. 1976). Moreover, since the evaporation process itself was found primarily responsible for viral inactivation during the air drying of sludge (Ward and Ashley 1977b), and digested sludge has been shown to air dry at a more rapid rate and to a greater extent than raw sludge (U.S. Environmental Protection Agency 1974), it follows that viral inactivation would probably be greater in anaerobically digested sludge than in raw sludge. Ward and Ashley (1978b) also observed the inactivation rate of poliovirus type 1 (CHAT) seeded in dewatered raw sludge to increase with a corresponding increase in temperature. Consequently, viral inactivation is likely to be substantially accelerated during the air drying of sludge at higher temperatures than the 21°C employed by Ward and Ashley (1977b). Under the most ideal conditions, however, sludge dewatering by air drying in sandbeds is not likely to yield a virus-free product. In Florida, for example, Wellings et al. (1976a) detected 24 PFU of echovirus type 7 in 250 grams of air-dried (for 13 days) sludge obtained from drying beds. Due to the possible viral hazard, all air-dried sludges should be handled with care during further treatment and final disposal (Wellings et al. 1976a).

Dewatering-drying lagoons. Lagoons have also been commonly used in the United States for sludge dewatering (see Figure 2-1) (Fair et al. 1968; U.S. Environmental Protection Agency 1974). Bitton (1980b) reported that 264,000 dry tons of treated sludge (or 4.5% of the total sludge available) are discharged yearly into lagoons in the United

States. Due to the great potential for odor problems associated with sludge lagoons, only well-stabilized sludge has been recommended for dewatering in lagoons (U.S. Environmental Protection Agency 1974). Criteria for the design of drying lagoons can be found in the literature (Sanks et al. 1976; U.S. Environmental Protection Agency 1974). Particularly important are the design criteria intended for the protection of groundwater supplies. For example, the bottom of sludge lagoons must be at least 18 inches above the maximum groundwater table in order to prevent groundwater contamination (U.S. Environmental Protection Agency 1974). In drying lagoons,water removal from sludge is primarily achieved by evaporation (U.S. Environmental Protection Agency 1974). Therefore, the effectiveness of sludge dewatering in drying lagoons is mostly influenced by weather conditions (e.g., maximum drying rate in hot, arid climate) and by sludge depth (e.g., drying rate increases as the sludge depth decreases) (U.S. Environmental Protection Agency 1974). The little information available indicates that sludge dewatering in drying lagoons is an extremely slow process. Sludge held in a lagoon at depths of 2 to 4 feet, for example, required three years to dewater from 5% solids content to 45% solids content (U.S. Environmental Protection Agency 1974).

Long-term lagooning has been found to destroy a significant fraction of the pathogens in digested sludge (U.S. Environmental Protection Agency 1978b). In lagoons that had stopped receiving additional quantities of digested sludge prior to their investigations, Sattar and Westwood (1979) and Farrah et al. (1981a) confirmed that indigenous enteric viruses associated with lagooned sludge are inactivated at a measurable rate. Under the warm temperatures of late

spring in Florida, Farrah et al. (1981a) found, for example, that the enterovirus titer of lagooned sludge dropped from 80 $TCID_{50}/g$ of dry sludge to low or undetectable levels in approximately 6 weeks (note that similar decline was also observed for fecal coliforms in the lagooned sludge). In contrast, Sattar and Westwood (1979) detected enteric viruses in 39% of the sludge samples obtained from a lagoon in Ottawa, Canada, over a 14-month period (i.e., from April 1975 to May 1976). These investigators were able to recover viruses from sludge samples taken from the lagoon after 8 months. Clearly, indigenous viruses were inactivated at a much slower rate in the Canadian sludge lagoon (Sattar and Westwood 1979) than in the Floridian sludge lagoon (Farrah et al. 1981a). Apparently, lower Ottawa temperatures (Sattar and Westwood 1979) contributed to greater viral persistence in the Canadian sludge lagoon. Whereas temperature appears to be an important factor affecting the inactivation rate of viruses in sludge lagoons, sludge drying is unlikely to have a significant effect. This is because, in lagoons, sludge dries at such a slow rate that substantial viral inactivation cannot be expected to result from the drying process itself. In addition to sludge treatment (i.e., drying and further digestion), lagoons also provide a temporary method of sludge storage (Fair et al. 1968; U.S. Environmental Protection Agency 1974). Ultimately, however, lagooned sludge must be disposed of and the method of choice is usually land application (U.S. Environmental Protection Agency 1974). Although the research presented above indicates that long-term

lagooning substantially reduces the viral content of digested sludge, indigenous enteric viruses have, nevertheless, been routinely detected in lagooned sludge undergoing land application (Farrah et al. 1981a; Sattar and Westwood 1979; Turk et al. 1980). Consequently, all lagooned sludges should be handled with care during final disposal in order to avoid possible viral hazards (Sattar and Westwood 1979).

Heat drying and reduction. Numerous heat drying and reduction processes (see Figure 2-1) are currently used for the removal of water from and for the reduction in the volume of sludge (U.S. Environmental Protection Agency 1974, 1978b). Reduction processes also destroy a major portion of sludge solids (U.S. Envrionmental Protection Agency 1974). Due to the extremely high temperatures employed in these treatment processes, all pathogens, including enteric viruses, in sludge are destroyed (U.S. Environmental Protection Agency 1974, 1978b). Rising energy costs, however, are making these sludge treatment processes impractical (U.S. Environmental Protection Agency 1974, 1978b). It should be pointed out that the heat-dried sludge or ash produced by the drying or reduction processes, respectively, require final disposal.

Sludge irradiation. The treatment of sludge with ionizing radiation has recently been shown to be highly effective in destroying the pathogens, including enteric viruses, present in sludge (see review by Bitton 1980b). In particular, thermoradiation (i.e., ionizing radiation combined with moderate heat) has been demonstrated by Ward (1977) to rapidly inactivate poliovirus type 1 (CHAT) seeded in raw

sludge. The combined heat and radiation treatments appeared to have a synergistic effect on the survival of poliovirus in raw sludge (Ward 1977). Due to its usual lack of pathogens, irradiated sludge has been recommended for use as an animal feed supplement or for other agricultural purposes (Bitton 1980b). It is worth noting, however, that sludge irradiation is an energy-intensive process that has yet to become widely used in the treatment of sludge (note that it is not included in Figure 2-1 as a standard sludge treatment process).

Final sludge disposal. Methods for final sludge disposal will be dealt with in a subsequent section.

Viral and Other Health Hazards Associated with Treated Sludges

As described above, most sludge treatment processes do not yield a virus-free product. With the possible exception of sludges treated at high temperatures, treated sludges represent potential health hazards due to the presence of viral pathogens (see review presented above). In addition to viruses, treated sludges (e.g., digested sludges) also contain bacterial pathogens (Foster and Engelbrecht 1973; Kowal and Pahren 1978), parasites (Hays 1977; Kowal and Pahren 1978; Little 1980; Pahren et al. 1979), toxic metals such as cadmium, copper, nickel, lead, zinc, and chromium (Chaney 1980; Jones and Lee 1978; Kowal and Pahren 1978; Pahren et al. 1979), and toxic organic residues such as aldrin, dieldrin, chlordane, heptachlor, lindane, toxaphene, polychlorinated biphenyls, and benzo(a)pyrene (Dacre 1980; Jones and Lee 1978; Kowal and Pahren 1978; Pahren et al. 1979). Clearly, the biological

and chemical properties of sludge are quite complex (Peterson et al. 1973; U.S. Environmental Protection Agency 1978b). Due to the numerous health hazards associated with treated sludges, final sludge disposal should be handled with the utmost of care.

Final Sludge Disposal

In 1979, municipal treatment plants in the United States were producing approximately 4.5 billion dry kg of sludge per year and this is expected to rise to 8 billion by the early 1980s (Pahren et al. 1979). Such large quantities of sludge coupled with the health hazards associated with sludge make final sludge disposal the most difficult of the sludge treatment processes (see Figure 2-1). Final sludge disposal is usually accomplished by either ocean dumping, sanitary landfill or land application (see Figure 2-1) (U.S. Environmental Protection Agency 1974, 1978b). Sludge incineration is not technically a final disposal method since ash is produced which requires disposal (in Figure 2-1, incineration is classified as a sludge reduction process). In actual practice, however, sludge incineration is considered a disposal method (Pahren 1980; Pahren et al. 1979; U.S. Environmental Protection Agency 1978b).

Incineration. Of the total amount of sludge disposed of in 1979 nationally, approximately 35% was incinerated (Pahren et al. 1979). In the future, however, sludge disposal by incineration is likely to be significantly curtailed due to air pollution, high energy costs and other problems associated with this disposal practice (Pahren 1980; Pahren et al. 1979; U.S. Environmental Protection Agency 1974, 1978b).

Moreover, sludge incineration destroys a potentially valuable resource which could be utilized, for example, on agricultural land (Pahren 1980).

Ocean dumping. For years, seacoast communities have been discharging digested sludge offshore in deep water (Fair et al. 1968; U.S. Environmental Protection Agency 1974). Approximately 15% of the sludge disposed of in 1979 nationally was dumped in the ocean (Pahren et al. 1979). By the end of 1981, however, ocean disposal of sludge will be prohibited by the U.S. federal government (Cowlishaw and Roland 1973; Pahren 1980; Pahren et al. 1979; U.S. Environmental Protection Agency 1974). The primary reason for prohibiting ocean dumping is that such a practice has been found to have long-term adverse effects on the ocean environment and on marine life (Cowlishaw and Roland 1973).

Sanitary landfill. The burial of sludge (i.e., sludge covered by a soil depth greater than the plow layer) in a sanitary landfill is another popular and acceptable method for sludge disposal (U.S. Environmental Protection Agency 1974, 1978b). Of the total amount of sludge disposed of in 1979 nationally, approximately 25% was buried in sanitary landfills (Pahren et al. 1979). In order to prevent odor, pathogen, operational and other problems, disposal in sanitary landfills is usually restricted to well-stabilized, dewatered ($\geq$15% solids content for sludge-only landfills) sludge (U.S. Environmental Protection Agency 1974, 1978b). The disposal of sludge in improperly managed landfills can result in groundwater pollution (Pahren 1980).

Land application. Land application of sludge is receiving increased attention and will probably be the predominant sludge

disposal method of the future (U.S. Environmental Protection Agency 1974, 1978b). Of the total amount of sludge disposed of in 1979 nationally, approximately 25% was applied to land (Pahren et al. 1979). In addition to achieving the goal of disposal, the application of sludge to strip-mined land and to cropland provides resource utilization in land reclamation and crop production, respectively (U.S. Environmental Protection Agency 1974, 1978a, 1978b).

Sludge application benefits cropland in several ways. Nutrients which are abundantly present in municipal sludge are utilized effectively by growing plants (Cowlishaw and Roland 1973; Pahren 1980; Pahren et al. 1979; U.S. Environmental Protection Agency 1974, 1978a, 1978b). Sludge application also improves several soil properties which are important for crop growth. For example, sludge addition increases the water content, water retention capacity, cation exchange capacity (CEC), organic carbon content and stable aggregate content of soils (Epstein 1975; Epstein et al. 1976). The application of lime-stabilized sludge increases soil pH and can, therefore, increase significantly the productivity of acidic soils such as those found in many humid regions (Brady 1974). Land application of sludge involving resource utilization (e.g., cropland application) has distinct advantages over disposal-only methods and is favored by the U.S. Environmental Protection Agency (Pahren 1980; U.S. Environmental Protection Agency 1978a). It has been estimated that only 1.3% of cultivated lands would be required for the application of all the sludge and animal waste produced in the United States (U.S. Environmental Protection Agency 1978b).

The practice of applying sludge to land is not without its problems. Water movement, for example, has been found to be restricted (i.e., saturated hydraulic conductivity declined) in sludge-treated soils (Epstein 1975). Such a reduction in the soil conductivity has been attributed to the clogging of soil pores by microbial decomposition products (Epstein 1975). If improperly applied, sludge has been found to inhibit the growth of a previously planted crop. For example, lime-stabilized sludge was reported to form a filamentous mat on the soil surface which resulted in the partial inhibition of previously planted wheat (U.S. Environmental Protection Agency 1978a). No matting or crop inhibition was observed when lime-stabilized sludge was incorporated into the soil prior to planting (U.S. Environmental Protection Agency 1978a).

Undoubtedly, the major drawback of sludge application to land is the possible dissemination of pathogens and toxic chemicals leading to adverse effects on human and animal life (Burge and Marsh 1978; Elliott and Ellis 1977; Foster and Engelbrecht 1973; Kowal and Pahren 1978; Pahren 1980; Pahren et al. 1979). In particular, enteric viruses present in sludge could potentially move through the soil matrix and contaminate groundwater supplies (see reviews by Berg 1973b; Bitton 1975; Bitton 1980a; Bitton et al. 1979b; Burge and Marsh 1978; Burge and Parr 1980; Cliver 1976; Duboise et al. 1979; Elliott and Ellis 1977; Foster and Engelbrecht 1973; Gerba et al. 1975; Moore et al. 1978; Sagik 1975). Due to the poor mixing and slow flow (generally <1 ft /day) conditions found in aquifers, many pollutants entering the

groundwater environment are not appreciably diluted and can persist for long periods of time (Lee 1976).

Although sludge can be applied to land in several forms (i.e., liquid, dewatered or cake-dried), the application of sludge in the liquid form is usually preferred because of its simplicity (U.S. Environmental Protection Agency 1974, 1978b). For example, dewatering processes are not required and inexpensive transfer systems (e.g., tank trucks) can be employed for handling liquid sludges (U.S. Environmental Protection Agency 1978b). Liquid sludge is usually applied to land using one of the following methods: spray irrigation, surface spreading--ridge and furrow irrigation, surface spreading--followed by sludge incorporation into the topsoil within 2 to 14 days, and subsurface injection (U.S. Environmental Protection Agency 1974, 1978b). There are potential problems associated with each of these application methods. While spray irrigation is a flexible method that requires minimum soil preparation, dangerous aerosols containing pathogens are generated during the spraying of sludge (U.S. Environmental Protection Agency 1974). The spreading of sludge on the soil surface can lead to the contamination of surface waters via runoff and/or soil erosion (U.S. Environmental Protection Agency 1974). However, if the applied sludge is promptly incorporated into the topsoil, surface water pollution, odor and aesthetic problems are largely eliminated (U.S. Environmental Protection Agency 1974). The injection of sludge below the soil surface avoids many of the problems associated with other application methods (e.g., aerosols, runoff and odors) (U.S. Environmental Protection Agency

1974). Due to the more favorable subsurface environment, however, pathogens (e.g., viruses) may persist longer in subsurface-injected sludge than in surface-applied sludge (Moore et al. 1978).

Whatever the application method employed, the disposal of sludge on land should be undertaken in accordance with local, state, and federal regulations and recommendations (Manson and Merritt 1975; U.S. Environmental Protection Agency 1974, 1978b; Wright 1975). Generally, only stabilized sludge is recommended for land application (U.S. Environmental Protection Agency 1974). Application rates of sludge to cropland vary depending upon sludge composition, soil characteristics, climate, vegetation and cropping practices (U.S. Environmental Protection Agency 1974), but should not exceed 20 dry tons of sludge solids/acre/year (44.8 dry metric tons/ha/year) or 46.8 m^3/ha/day (liquid rate) (Manson and Merritt 1975; U.S. Environmental Protection Agency 1974). In order to prevent groundwater and surface water contamination, as well as other potential problems, Manson and Merritt (1975) recommended that sludge disposal sites conform to the following standards:

1. High water tables should be no closer to the soil surface than 4 ft (1.22 m)
2. Isolation from surface waters by a minimum distance of 200 ft (61 m)
3. Maximum slope of 5% in order to prevent excessive surface runoff
4. A crop that can be harvested is the preferred ground cover

5. A minimum distance of 250 ft (ca. 76 m) to the nearest residence
6. Access to the sludge disposal site should be restricted

Sludge disposal on properly managed sites in the United States has been successful and has not led to significant problems (Manson and Merritt 1975; U.S. Environmental Protection Agency 1974).

Fate of Sludge-Associated Viruses in Soils

Literature pertaining to the survival and possible movement of enteric viruses in sludge-treated soils is presented in the Introductions to Chapters IV through VI.

CHAPTER III
EFFECT OF SLUDGE TYPE ON POLIOVIRUS ASSOCIATION WITH AND RECOVERY FROM SLUDGE SOLIDS

Introduction

The degree of association between viruses and sludge solids is a critically important factor in the assessment of the fate of these pathogens following sludge disposal on land. Yet, the nature of the association between viruses and sludge solids has not been adequately explored, partly because of the lack of virological methods. Recently, however, methods have been developed for the recovery of viruses from wastewater sludges. Practical methods for the recovery of viruses from sludge samples involve two steps. Because viruses in sludges have been found to be solids associated (Abid et al. 1978; Glass et al. 1978; Hurst et al. 1978; Lund 1971; Ward and Ashley 1976; Wellings et al. 1976a), the first step of an effective method, therefore, consists of releasing both surface-adsorbed and solids-embedded viruses. The second step consists of concentrating the eluted viruses prior to viral assays.

Various chemicals have been used to elute viruses from sludge solids, namely 0.1% sodium lauryl sulfate in 0.05 M glycine, pH 7.5 (Abid et al. 1978), tryptose phosphate broth (Moore et al. 1978), 3% beef extract, pH 9.0 (Wellings et al. 1976a), 3% beef extract, ambient pH (Glass et al. 1978; Sattar and Westwood 1976), 0.05 M glycine buffer, pH 11.0 (Hurst et al. 1978), 2% fetal calf serum in Earle's

balanced salt solution, pH 9.5 (Subrahmanyan 1977), and 10% fetal calf serum (Sattar and Westwood 1976). Elution of solids-associated viruses may be aided by sonication (Abid et al. 1978; Glass et al. 1978; Wellings et al. 1976a), shaking on a wrist-action shaker (Sattar and Westwood 1976), homogenization in a blender (Glass et al. 1978; Moore et al. 1978; Subrahmanyan 1977), or magnetic stirring (Abid et al. 1978; Hurst et al. 1978). Eluted viruses have been concentrated by organic flocculation at low pH (Abid et al. 1978; Glass et al. 1978; Hurst et al. 1978), hydroextraction (Wellings et al. 1976a) or adsorption to bentonite clay (Turk et al. 1980).

Several of the methods proposed for the recovery of viruses from sludges do not contain a concentration step but simply involve the elution of viruses from sludge solids (Moore et al. 1978; Sattar and Westwood 1976; Subrahmanyan 1977). The use of these methods is limited to raw sludges and other sludges containing large amounts of viruses. The concentration method proposed by Wellings et al. (1976a) involving hydroextraction is cumbersome and requires considerably more time to perform than organic flocculation. The procedure developed by Abid et al. (1978) is not practical, since it does not adequately concentrate the viruses eluted from sludge solids. Therefore, the best methods proposed to date for virus recovery from sludges appear to be those of Glass et al. (1978) and Hurst et al. (1978). Working with anaerobically digested sludge, Glass et al. (1978) obtained an overall recovery of poliovirus type 1 (CHAT) of 31%. Hurst et al. (1978) found that poliovirus type 1 (LSc) could be recovered from activated sludge

samples with an overall efficiency of 80%. It is difficult to compare these two methods, since they were evaluated with different sludge types.

The research reported in subsequent chapters of this dissertation deals primarily with the fate of enteroviruses following sludge disposal on land. In the course of conducting this research, the viral (i.e., indigenous and seeded) content of different sludge types had to be determined. Consequently, it was imperative to determine if viruses could be effectively recovered from the solids of different sludge types. The glycine method developed by Hurst et al. (1978) was evaluated for its effectiveness in recovering poliovirus type 1 (LSc) from different sludge types. The sludge types used were activated sludge mixed liquor, and anaerobically and aerobically digested sludges. It is the aim of this chapter to show that sludge type is a factor that can strongly influence the degree of association between viruses and sludge solids, as well as the recovery of sludge solids-associated viruses by the glycine method (Hurst et al. 1978).

Materials and Methods

Virus and Viral Assays

Poliovirus type 1 (strain LSc) was used in the research reported in this chapter. This virus strain is a non-neurotropic variant of the Mahoney strain, and is avirulent for mice and monkeys by all routes. This strain is identical to the virus designated as attenuated Sabin strain (Cooper 1967; Hahn 1972; World Health Organization 1968). Some general properties of polioviruses are shown in

Table 3-1. Stocks of the virus were prepared by infecting monolayer cultures of AV3 (a continuous line of human amnion), BGM (a continuous line of Buffalo green monkey kidney--Barron et al. 1970; Dahling et al. 1974) or MA-104 (a continuous line of fetal rhesus monkey kidney) cells in a 32 oz (128 cm^2) glass bottle (rubber-lined, screw-capped--Brockway Glass Co., Inc.). After allowing an adsorption period of 60 minutes with tilting at 15-minute intervals, the cells were overlaid with 40 ml of Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 250 U/ml penicillin, and 125 µg/ml streptomycin (see Appendix for more details on the composition of this and other media used). After approximately 48 hours of incubation at 37°C, the overlay medium was decanted and then centrifuged at 270 x g for 15 minutes at 4°C to remove cell debris. The resulting supernatant containing the virus was distributed in 1, 2 or 5 ml aliquots and immediately frozen at -70°C. The virus was kept at -70°C until used.

Poliovirus was assayed by the plaque technique (Cooper 1967) on AV3, BGM or MA-104 cell monolayers prepared as follows. Confluent cell monolayers were grown in 32 oz glass bottles using Eagle's MEM supplemented with 10% FCS, 250 U/ml penicillin and 125 µg/ml streptomycin (i.e., growth medium; see Appendix). Each cell monolayer was then washed three times with 10 ml of the pre-trypsin solution (see Appendix). This treatment removes all traces of serum (contains trypsin inhibitors), as well as Ca^{+2} and Mg^{+2} ions (enhance adsorption of cells to glass). To remove the cells from the glass bottle, 10 ml of the standard trypsin-versene solution (see Appendix) was then added

TABLE 3-1. General properties of polioviruses

Property	Value
Nucleic acid	RNA[a,b] (single-stranded)
Molecular weight of nucleic acid (daltons)	2×10^6[a,b]
Particle diameter (nm)	27 to 30[a,b,c]
Particle morphology	Icosahedral[a,b]
Particle isoelectric point	4.5 and 7.0[d]
Stability at 25°C	Relatively stable[a]
Stability at pH 3.0	Stable[a,b,e]
Stability in ether	Stable[b]

[a]From Davis et al. (1973).

[b]From Hahn (1972).

[c]From Schwerdt and Schaffer (1955).

[d]From Mandel (1971). The data were obtained using poliovirus type 1 (strain Brunhilde).

[e]From Bachrach and Schwerdt (1952).

and allowed to spread over the entire monolayer for 30 to 60 seconds. This solution was subsequently decanted and the cell culture was allowed to rest at room temperature until the cells came off the glass (approximately 5 minutes). Growth medium (10 ml) was then added, and pipetted twice up and down to dislodge the cells from the glass and break up clumps. An additional 190 ml of the growth medium was added to the content of the 32 oz glass bottle. This cell suspension was then distributed in 5 ml aliquots to 40 glass (2 oz--20 cm^2) or plastic (25 cm^2) bottles. After approximately 48 hours of incubation at 37°C, these small bottles contained confluent cell monolayers and were ready for use in viral assays.

Experimental samples were diluted, if necessary, prior to assay in either Eagle's MEM containing 5% calf serum (rarely used) or phosphate-buffered saline (PBS) containing 2% FCS (both solutions contained 250 U/ml penicillin, 125 µg/ml streptomycin, and phenol red; see Appendix). All samples from each experiment were assayed on only one cell line (i.e., AV3, BGM or MA-104) using the procedure described below. A 0.6 ml aliquot of each diluted or undiluted sample was inoculated in fractions of 0.2 ml into three drained cell monolayers. Following inoculation, a 60-minute adsorption period with tilting at 15-minute intervals was allowed. The infected monolayers were then overlaid with 4 ml of 1% methyl cellulose in Eagle's MEM supplemented with 5% FCS, 250 U/ml penicillin, 125 µg/ml streptomycin, and 117 µg/ml kanamycin (see Appendix). After incubation at 37°C for approximately 48 hours, the cell monolayers were stained with either crystal violet

or neutral red (see Appendix). Plaques were subsequently counted with the unaided eye as suggested by Cooper (1967). In several experiments, the plaques were counted using an Omega photographic enlarger B22 (Simmon Brothers, Woodside, New York). Each tabulated viral count represents the average of triplicate counts. The numbers of viruses were expressed as plaque-forming units (PFU).

Sludges

A variety of wastewater sludges was used in this study as listed in Table 3-2. The sludges were obtained from four wastewater treatment plants located in Gainesville and Pensacola, Florida. In addition, a lagooned sludge sampled at the West Florida Agricultural Experiment Station (Jay, Florida) was also used. The treatments the sludges received before being sampled are also shown in Table 3-2. One of the sludge types used was activated sludge mixed liquor. The term "mixed liquor" refers to the suspension undergoing treatment in an activated sludge unit. The abbreviated sludge designations (see Table 3-2) will be used to identify sludges in the rest of this dissertation. The sludges were collected in sterile Nalgene bottles, transported to the University of Florida (Gainesville) laboratory and then immediately refrigerated. All sludge samples were used within 30 days of sampling and most samples were used within 3 days. At the time of use, a sludge sample was first allowed to come to room temperature. The pH and solids content of the sludge was then determined. The pH was measured using a digital pH meter model 125 from Corning (Corning, New York). The solids content was determined by drying in an oven at 105°C

TABLE 3-2. Sources of the wastewater sludges used in this study

Wastewater treatment plant[a] generating the sludge	Abbreviated sludge designation	Sludge[b] type used	Sludge treatment		
			Digestion procedure	Digestion time (days)	Additional treatment
Campus plant (Univ. of Florida)	UML	Mixed liquor[c]	--[d]	--	--
	UDA	Digested	Aerobic	39	--
Main street plant (Gainesville, Florida)	GML	Mixed liquor[c]	--	--	--
	GDA90	Digested	Aerobic	90	--
	GDA180	Digested	Aerobic	180	--
	GDAN	Digested	Anaerobic	60	--
Montclair plant (Pensacola, Florida)	PDA	Digested (and dewatered)	Aerobic	30	Conditioned with Magnafloc[e] 1563c and then dewatered
Main street plant (Pensacola, Florida)	PDAN	Digested (and dewatered)	Anaerobic	60	Conditioned with Magnafloc[e] 2535c and then dewatered
Montclair and Main street plants (Pensacola, Florida)	LAG	Lagoon[f]	--	--	--

[a]All plants treated municipal wastewater with the exception of the Main street plant of Pensacola which treated a mixture of municipal (2/3) and industrial (1/3) wastewater.

[b]The sludges used were generated during the secondary treatment (activated sludge or tricking filter) of wastewater.

[c]The term "mixed liquor" refers to the suspension undergoing treatment in an activated sludge unit. Although they contain activated sludge solids, mixed liquors are not to be confused with settled sludges which must be treated and ultimately disposed of.

[d]A dash means that the sludge treatment was either not applicable or not performed.

[e]These are cationic polymers supplied to the treatment plants by the American Cyanamid Company, Wayne, N.J.

[f]This sludge is a mixture of sludge from the Montclair (1/3) and Main street (2/3) plants of Pensacola, Florida. The mixture was kept in a lagoon at the West Florida Agricultural Experiment Station (Jay, Florida) before ultimately being disposed of on land. It was the lagooned sludge which was sampled and used in this study.

for 24 hours a measured volume of sludge and was expressed as a percentage on a weight (grams) to volume (milliliters) basis. Most of the sludges used were not autoclaved or decontaminated in any other way. Due to uncontrollable contamination of cell monolayers during viral assays, two sludge samples (GDAN and PDAN--see Table 3-2) had to be sterilized by autoclaving at 121°C with applied pressure of 15 psi for 15 minutes prior to use. During sludge treatment, digested sludges are frequently subjected to heat (i.e., 150 to 260°C under pressures of 150 to 400 psi) as a conditioning step which improves subsequent sludge dewatering (U.S. Environmental Protection Agency 1978a; also see Figure 2-1). Thus, autoclaved sludge can be considered to be heat-conditioned sludge. As seen in Figure 3-1, the solids of autoclaved, anaerobically digested sludge (GDAN--see Table 3-2) settled in 2 hours while those of nonautoclaved sludge remained dispersed. This increase in the rate of settling of sludge solids probably accounts for the improved-dewatering property of heat-conditioned sludge. Apart from the effect on the rate of settling of sludge solids, autoclaving probably did not significantly alter other sludge properties which affect sludge-virus interactions. For example, Ward and Ashley (1977a) found that autoclaving does not destroy the enterovirus-inactivating capacity of anaerobically digested sludge. Furthermore, autoclaving did not affect the degree of association between poliovirus and anaerobically digested sludge solids as determined below.

Association of Seeded Poliovirus with Sludge Solids

One milliliter of poliovirus stock in PBS containing 2% FCS was added directly to 100, 500, or 1,000 ml of sludge while stirring the

FIGURE 3-1. Effect of autoclaving on the rate of settling of anaerobically digested sludge solids

An aliquot of anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3,250 μmho/cm at 25°C and 8.3, respectively) was autoclaved at 121°C with applied pressure of 15 psi for 15 minutes and compared to an aliquot of the sludge which had not been autoclaved. Approximately 21 ml each of autoclaved sludge and nonautoclaved sludge were added to graduated cylinders A and B, respectively. After 2 hours, the solids of the autoclaved sludge had settled while those of the nonautoclaved sludge remained dispersed.

A B

suspension using either a magnetic stirrer or pipette (magnetic stirring could not be used for some sludges which had high solids contents). Poliovirus was seeded in the sludge samples at concentrations approximately 1,000 times greater than the indigenous virus levels measured in the sludges used. Therefore, indigenous viruses present in the sludge samples did not affect the results presented herein. Magnetic stirring (or frequent mixing with a pipette) was continued for 10 minutes to 60 minutes. Following the contact period, an aliquot of the unfractionated sludge (i.e., sludge sample without solids separated) was diluted in PBS containing 2% FCS and assayed directly for seeded viruses by the plaque technique. This assay was performed in order to determine if the direct viral assay after the contact period would agree with the calculated virus input based on the added volume of poliovirus stock of known titer. From Table 3-3, it can be seen that poliovirus was recovered with a mean efficiency of 109%, 104%, and 98% from mixed liquors, aerobically digested sludges and anaerobically digested sludges, respectively, following dilution and subsequent direct assay on cell cultures. Thus, poliovirus type 1 (LSc) added to sludge was recovered effectively after a contact period of 10 to 60 minutes without any significant inactivation. Direct inoculation of unfractionated sludge into cell cultures has been found to be toxic to cells (Nielsen and Lydholm 1980; Subrahmanyan 1977). In my research, the diluted sludge samples were not toxic to the cell cultures. Similarly, Hurst et al. (1978) diluted virus-seeded activated sludge in Tris buffer and then successfully inoculated it directly into cell cultures without causing cell toxicity. Some of the sludges used in my

TABLE 3-3. Recovery of poliovirus type 1 from unfractionated sludge by dilution and subsequent direct assay on cell cultures

Sludge type	Sludges used	No. of experimental trials	Calculated virus[a] input (mean, total PFU)	Mean recovery[b] of calculated virus input (% ± SE[c])
Mixed liquor	UML, and GML	2	4.8×10^6	108.9 ± 6.6
Aerobically digested	UDA, PDA, GDA90, and GDA180	11	8.2×10^7	103.6 ± 15.7
Anaerobically digested	GDAN, PDAN, and LAG[d]	10	2.1×10^8	98.1 ± 9.0

[a]Poliovirus was added to 1000 ml of sludge while stirring the mixture using a magnetic stirrer. The calculated virus input was based on the volume of poliovirus stock of known titer added to the sludge. Magnetic stirring was continued for 10 min to 60 min.

[b]Following the contact period, an aliquot of the unfractionated sludge (i.e., the sludge solids were not separated by centrifugation) was diluted in PBS containing 2% FCS, 250 U of penicillin per ml, 125 µg of streptomycin per ml and phenol red, and assayed by direct inoculation into cell cultures.

[c]Abbreviation for standard error.

[d]The lagoon sludge is a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3), and consequently, its properties are close to those of anaerobically digested sludges (see Table 3-2). Therefore, lagoon sludges were placed in the population of anaerobically digested sludges.

study did, however, produce cell culture contamination when diluted and then directly inoculated into cell cultures. For these sludges (i.e., when not decontaminated by autoclaving), the initial virus present was determined based on the amount of virus stock of known titer added to the sludge. From the results shown in Table 3-3, it is believed that the determination of the initial virus added to the sludge was accurately achieved by either direct viral assay of the sludge or based on the amount of virus stock of known titer added to the sludge. The total sludge volume was subsequently centrifuged at 1,400 x g for 10 minutes at 4°C (only an aliquot of sludges GDA180 and GDAN was centrifuged). An aliquot of the sludge supernatant produced was assayed for viruses. Thus, allowing the calculation of the "viable unadsorbed virus" fraction as shown below:

$$\text{viable unadsorbed virus (\%)} = \frac{\text{virus in sludge supernatant (total PFU)}}{\text{virus in unfractionated sludge (total PFU)}} \times 100 \qquad (3\text{-}1)$$

Furthermore, the "sludge solids-associated virus" fraction was also estimated as shown below:

$$\text{solids-associated virus (\%)} = 100 - \text{viable unadsorbed virus (\%)} \qquad (3\text{-}2)$$

For sludges GDA180 and GDAN, viral assays were performed, as described above (i.e., of unfractionated sludge and of sludge supernatant), several times throughout a 12-hour to 8-day period. During this period, the virus-seeded sludge was left at room temperature undisturbed, and was stirred only prior to obtaining a sample for viral assay.

Recovery of Seeded Poliovirus from Sludge Components

The sludge supernatant and sludge solids generated were separately subjected to the virus recovery methodology described below. In several experiments, the sludge supernatants were not processed for virus concentration. These supernatants were simply adjusted to neutral pH and assayed for viruses as described previously. The number of viruses thus found were included in the "overall virus recovery" values reported. In those experiments in which the sludge supernatants were subjected to the virus concentration procedure, the supernatants were processed like the sludge solids eluates described below.

The sludge solids-associated viruses were eluted and further concentrated using a modification of the glycine method developed by Hurst et al. (1978). The solids were mixed with five volumes of 0.05 M glycine buffer, pH 11.5. The pH of the mixture was adjusted to between 10.5 and 11.0 by the addition of 1 M glycine buffer, pH 11.5. The samples were vigorously mixed for 30 seconds using a magnetic stirrer and centrifuged at 1,400 x g for 5 minutes at 4°C (all centrifugation was performed using a Sorvall RC5-B centrifuge, Ivan Sorvall Inc., Norwalk, Connecticut). The supernatants (i.e., the sludge solids eluates) were recovered, adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for eluted viruses. The entire procedure described above was performed in less than 10 minutes. Thus, poliovirus was subjected to the high pH of 10.5 to 11.0 for no more than 10 minutes. Both Hurst et al. (1978) and Sobsey et al. (1980b) observed no appreciable inactivation in 10 minutes of poliovirus type 1

(LSc) seeded in 0.05 M glycine buffer, pH 10.5 to 11.0. Therefore, it is believed that there was no significant inactivation of poliovirus during the elution procedure. It should be noted, however, that this elution method is not practical for the recovery of reoviruses and rotaviruses from sludge. These virus types are rapidly inactivated when subjected to such high pH values (Sobsey et al. 1980b). The viruses in the sludge solids eluates were concentrated by organic flocculation (Katzenelson et al. 1976b) as follows. The eluates were adjusted to pH 3.5 by the addition of 1 M glycine buffer, pH 2.0, and the flocs produced were pelleted by centrifugation at 1,400 x g for 20 minutes at 4°C. The supernatants and pellets produced were treated separately. The supernatants were assayed for viruses and then passed through a series of 3.0, 0.45, and 0.25 µm Filterite filters (Filterite Corp., Timonium, Maryland) in a 47-mm holder. Adsorbed viruses were eluted from the filters with 7 ml of PBS containing 10% FCS, pH 9.0. The filter eluates were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for viruses. The filtrates (i.e., the fluids having passed the filters) were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 11.5, and assayed for viruses. The pellets previously obtained by centrifuging the samples at pH 3.5 were mixed with five volumes of PBS containing 10% FCS, pH 9.0. The mixtures were adjusted to pH 9.0 by the addition of 1 M glycine buffer, pH 11.5, vortexed for 30 seconds and then centrifuged at 14,000 x g for 10 minutes at 4°C. The supernatants were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for viruses.

Viral assays were performed at various steps in the procedure in order to determine the efficiency of the individual steps. Each "overall virus recovery" value reported was determined from the viruses recovered in the filter concentrate, pellet concentrate and sludge supernatant (concentrated or not).

Statistical Treatment of Data

Statistical treatment of the data was performed with the use of a Hewlett-Packard calculator model 9810A and Statistics Package V-6 (Hewlett-Packard Company, Loveland, Colorado).

Results and Discussion

Association of Seeded Poliovirus with Sludge Solids

Poliovirus seeded in the various sludge samples rapidly became associated with the sludge solids. However, no statistically significant linear correlation was found between the percent solids contents of the sludges studied and the degree of virus association by the sludge solids (Table 3-4). This lack of correlation was found within each sludge type and for all sludge types combined. Thus, the sludge solids content was shown not to affect the association of virus with sludge solids, at least in the range of solids contents studied (i.e., 0.5% through 2.9%). This allowed sludges of different solids contents, but belonging to the same sludge type, to be grouped together in the same category.

The mean percent of solids-associated viruses for activated sludge mixed liquors, anaerobically digested sludges, and aerobically

TABLE 3-4. Effect of sludge type on the association between poliovirus type 1 and sludge solids

Sludge type	Sludge used	Sludge parameters		Virus[b] in unfractionated[c] sludge (total PFU)	Virus in sludge supernatant[d] (total PFU)	Viable unadsorbed[e] virus (%)	Solids-associated[f] virus (%)	Mean[g] associated virus for each sludge type (% ± SE[h])
		pH	Solids[a] content (%)					
Mixed liquor	UML	6.4	1.6	8.4×10^6	5.0×10^6	59.5	40.5	57 ± 17[A]
	GML	6.9	0.5	1.3×10^6	3.4×10^5	26.2	73.8	
Aerobically digested	UDA	4.8	1.5	6.5×10^6	1.1×10^6	16.9	83.1	95 ± 2.0[B]
	UDA	4.8	1.5	6.5×10^6	6.4×10^5	9.8	90.2	
	UDA	6.1	1.3	2.0×10^7	1.3×10^6	6.5	93.5	
	UDA	6.1	1.3	2.0×10^7	9.6×10^5	4.8	95.2	
	UDA	6.5	1.3	9.6×10^6	4.0×10^4	0.4	99.6	
	GDA90	5.8	1.0	1.3×10^7	4.3×10^5	3.3	96.7	
	GDA180	5.0	1.3	8.2×10^8	4.8×10^6	0.6	99.4	
	PDA	5.8	2.8	4.1×10^6	5.7×10^3	0.1	99.9	
Anaerobically digested	GDAN	8.3	2.0	1.0×10^7	4.4×10^6	44.0	56.0	70 ± 8.4[A]
	PDAN	7.2	1.4	6.0×10^6	3.2×10^6	53.3	46.7	
	PDAN	6.4	1.9	5.5×10^6	1.4×10^6	25.5	74.5	
	LAG[i]	7.3	2.9	1.8×10^6	2.9×10^5	16.1	83.9	
	LAG[i]	6.9	2.9	1.9×10^9	1.7×10^8	8.9	91.1	

[a]A two-tailed, t-test concerning the population correlation coefficient (r) revealed that at the 0.01 level there was no significant linear correlation (i.e., r = 0) between the solids content (%) of the sludge and the "solids-associated virus (%)" values. This lack of correlation was found within each sludge type and for all sludge types combined.

[b]The virus was added to the sludge while stirring the suspension using a magnetic stirrer. Magnetic stirring was continued for 10 to 60 min and then an aliquot of the virus-seeded sludge was obtained for viral assay.

[c]The sludge solids were not separated prior to assaying.

[d]The sludge was clarified by centrifugation at 1400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[e]The "viable unadsorbed virus (%)" values were calculated as shown in the Materials and Methods section.

[f]The "solids-associated virus (%)" values were estimated as shown in the Materials and Methods section.

[g]Mean values displaying different superscript capital letters are significantly different at the 0.01 level when subjected to analysis of variance using Duncan's test.

[h]Abbreviation for standard error.

[i]The lagoon sludge is a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3), and consequently, its properties are close to those of anaerobically digested sludges (see Table 3-2). Therefore, lagoon sludges were placed in the population of anaerobically digested sludges.

digested sludges was 57%, 70%, and 95%, respectively (Table 3-4). Ward and Ashley (1976) have obtained similar results with anaerobically digested sludge. These investigators have shown that 65% and 67% of seeded poliovirus type 1 (Mahoney) and poliovirus type 1 (CHAT), respectively, were associated with anaerobic sludge solids after a contact period of 15 minutes. The association between seeded poliovirus and sludge solids was significantly greater for aerobically digested sludges than for mixed liquors or anaerobically digested sludges. No statistically significant difference was found between the mean percent of solids-associated viruses for mixed liquors and anaerobically digested sludges. The lagoon sludges mentioned in Table 3-4 are a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3), and consequently, were placed in the category of anaerobically digested sludges. However, the association of poliovirus with lagoon sludge solids was greater than the association of this virus with other anaerobic sludge solids tested (see Table 3-4). Apparently, the presence of aerobic sludge solids (1/3) in lagoon sludge accounts for the greater ability of lagoon sludges to bind viruses.

The reason for the greater association of seeded viruses with aerobic sludge solids is still unknown. However, aerobically digested sludges generally displayed lower pH values (ranging from 4.8 to 6.5) than mixed liquors (6.4 and 6.9) or anaerobically digested sludges (ranging from 6.4 to 8.3) (see Table 3-4). Virus adsorption to surfaces is promoted at low pH. Other parameters of aerobically digested sludges, as yet unidentified, could also account for the greater ability of these

sludges to bind viruses. However, we have shown that the sludge solids content does not account for the differences observed in the virus-binding capacity of different sludge types. In the range of sludge solids contents studied, there appears to be sufficient sites for virus binding and, therefore, this sludge parameter is not a limiting factor in determining the association of viruses with sludge solids.

The degree of association between seeded poliovirus, and aerobic (see Table 3-5) or anaerobic (see Table 3-6) sludge solids remained fairly constant over a contact period of 8 days or 12 hours, respectively. Clearly, varying the contact time would not have significantly affected the results presented above on the virus-binding capacity of different sludge types. The inactivation rate of poliovirus seeded in sludge was also determined from the data shown in Tables 3-5 and 3-6. In anaerobically digested sludge, the total amount of infectious poliovirus present in the sludge (i.e., virus in unfractionated sludge) steadily declined over a 12-hour period (Table 3-6). In this sludge type, there was approximately a 50% reduction in the poliovirus titer in 12 hours (i.e., approximately 1 $\log_{10}$ reduction/36 hours) at room temperature (see Table-3-6). Ward and Ashley (1976) measured similar inactivation rates for poliovirus type 1 (CHAT and Mahoney) seeded in anaerobically digested sludge. In contrast to anaerobically digested sludge, there was no significant inactivation of poliovirus seeded in aerobically digested sludge during a 7-day contact period at room temperature (see Table 3-5). The low viral-inactivating capacity of aerobically digested sludge has also been

TABLE 3-5. Effect of contact time on the association between poliovirus type 1 and aerobically digested sludge solids

Contact time	Virus[a] in unfractionated[b] sludge (total PFU)	Virus in sludge supernatant[c] (total PFU)	Viable unadsorbed[d] virus (%)	Solids-associated[e] virus (%)
30 min	8.9×10^8	4.2×10^6	0.5	99.5
60 min	8.2×10^8	4.8×10^6	0.6	99.4
2 days	1.2×10^9	1.3×10^7	1.1	98.9
4 days	1.2×10^9	4.3×10^6	0.4	99.6
5 days	8.0×10^8	8.8×10^6	1.1	98.9
6 days	5.8×10^8	1.9×10^6	0.3	99.7
7 days	8.2×10^8	2.5×10^6	0.3	99.7
8 days	6.9×10^8	2.5×10^6	0.4	99.6

[a]The virus was added to 1000 ml of aerobically digested sludge (GDA180--see Table 3-2; solids content and pH equal to 1.3% and 5.0, respectively) while stirring the suspension using a magnetic stirrer. Magnetic stirring was continued for 60 min. For the remainder of the experimental trial, the virus-seeded sludge was left at room temperature undisturbed, and was stirred only prior to obtaining a sample for viral assay.

[b]The sludge solids were not separated prior to assaying.

[c]The sludge was clarified by centrifugation at 1400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[d]The "viable unadsorbed virus (%)" values were calculated as shown in the Materials and Methods section.

[e]The "solids-associated virus (%)" values were estimated as shown in the Materials and Methods section.

TABLE 3-6. Effect of contact time on the association between poliovirus type 1 and anaerobically digested sludge solids

Contact time (hours)	Virus[a] in unfractionated[b] sludge (total PFU)	Virus in sludge supernatant[c] (total PFU)	Viable unadsorbed[d] virus (%)	Solids-associated[e] virus (%)
0	1.1×10^7	4.6×10^6	41.8	58.2
0.5	1.1×10^7	3.5×10^6	31.8	68.2
1.0	1.0×10^7	4.4×10^6	44.0	56.0
6.0	9.4×10^6	2.9×10^6	30.9	69.1
8.5	6.5×10^6	2.3×10^6	35.4	64.6
12.0	5.3×10^6	2.5×10^6	47.2	52.8

[a]The virus was added to 1000 ml of anaerobically digested sludge (GDAN--see Table 3-2; solids content and pH equal to 2.0% and 8.3, respectively) while stirring the suspension using a magnetic stirrer. Magnetic stirring was continued for 60 min. For the remainder of the experimental trial, the virus-seeded sludge was left at room temperature undisturbed, and was stirred only prior to obtaining a sample for viral assay.

[b]The sludge solids were not separated prior to assaying.

[c]The sludge was clarified by centrifugation at 1400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[d]The "viable unadsorbed virus (%)" values were calculated as shown in the Materials and Methods section.

[e]The "solids-associated virus (%)" values were estimated as shown in the Materials and Methods section.

demonstrated in the field. Farrah et al. (1981a) found that aerobically digested sludge contained larger indigenous viral titers than anaerobically digested sludge. The uncharged form of ammonia has been shown to exist in sludge (i.e., tested raw and anaerobically digested sludge) mostly at pH values above 8 and to display virucidal activity against enteroviruses (Ward and Ashley 1976, 1977a). Due to the typically low pH of aerobically digested sludge (see Tables 3-4 and 3-5), it can be hypothesized that the virucidal ammonia is probably largely absent from this sludge type. Therefore, the low viral-inactivating activity observed in the aerobically digested sludge (GDA180 sludge, pH 5.0) employed in this study (see Table 3-5) was to be expected. Similarly, Ward and Ashley (1976) found no appreciable inactivation of seeded poliovirus type 1 in 5 days at 20°C in raw sludge maintained at its naturally low pH of 6.0. Naturally high pH values, on the other hand, have been found in anaerobically digested sludge and consequently, this sludge type has demonstrated substantial viral-inactivating activity due to the presence of the virucidal ammonia (Ward and Ashley 1976, 1977a). The results presented in Table 3-6 confirm that enteroviruses (i.e., used poliovirus type 1) seeded in anaerobically digested sludge (i.e., used GDAN sludge, pH 8.3) are inactivated at a significant rate.

It is emphasized that the results and conclusions presented above pertain only to the virus used, poliovirus type 1 (LSc). Other enterovirus may, in fact, display different patterns of adsorption to sludge solids. Research has shown that only 20.7% of seeded echovirus

type 1 (Farouk) became associated with lagoon sludge solids after a contact period of 60 minutes (see Table 3-4). From Table 3-4 it can be seen that a larger fraction of poliovirus type 1 (83.9% and 91.1%) became associated with lagoon sludge solids. Goyal and Gerba (1979) have also shown that seeded echovirus type 1 (Farouk) does not adsorb well to a sandy loam soil. It is clearly established that virus type is a factor that affects virus adsorption to surfaces, including sludge solids. It was the aim of this chapter, however, to show that sludge type is also a critical factor influencing the degree of association of viruses with sludge solids.

Recovery of Solids-Associated Viruses

Seeded viruses that became associated with sludge solids were eluted and further concentrated according to the glycine method. In Table 3-7, it can be seen that significantly lower mean poliovirus recovery was found for aerobically digested sludges (15%) than for mixed liquors or anaerobically digested sludges (72% and 60%, respectively). The mean poliovirus recoveries from mixed liquors and anaerobically digested sludges were not significantly different statistically. The recovery of solids-associated viruses was not dependent upon the volume of liquid sludge (100, 500, or 1,000 ml) processed. The mean poliovirus recovery from mixed liquors (72%) was similar to the recovery (80%) of the same virus reported by Hurst et al. (1978). These researchers worked with activated sludge, which is the same sludge type as our mixed liquors.

It is clearly established that the effectiveness of the glycine method in recovering solids-associated viruses is reduced for aerobically

TABLE 3-7. Effect of sludge type on the recovery of poliovirus type 1 from sludge using a modification of the glycine method

Sludge type	Sludge used	Sludge parameters		Volume of sludge processed[a] (ml)	Virus[b] added to sludge (total PFU)	Overall virus recovery[c] (%)	Mean[d] virus recovery for each sludge type (% ± SE[e])
		pH	Solids content (%)				
Mixed liquor	UML	6.4	1.6	100	8.4×10^6	84.3*[f]	72 ± 12[A]
	GML	6.9	0.5	1000	1.3×10^6	60.3	
Aerobically digested	UDA	4.8	1.5	500	6.5×10^6	18.9*	15. ± 2.7[B]
	UDA	4.8	1.5	500	6.5×10^6	11.9*	
	UDA	6.1	1.3	500	2.0×10^7	8.0*	
	UDA	6.1	1.3	500	2.0×10^7	5.9*	
	UDA	6.5	1.3	1000	9.6×10^6	15.5	
	GDA90	5.8	1.0	1000	1.3×10^7	14.1	
	PDA	5.8	2.8	1000	4.1×10^6	26.9	
Anaerobically digested	PDAN	7.2	1.4	100	6.0×10^6	59.9*	60 ± 2.1[A]
	PDAN	6.4	1.9	1000	5.5×10^6	63.9*	
	LAG[g]	7.3	2.9	1000	1.8×10^6	56.7	

[a]The procedure used was a modification of the method developed by Hurst et al. (1978) and is described in the Materials and Methods section.

[b]The virus was added to sludge while stirring the suspension using a magnetic stirrer. Magnetic stirring was continued for 10 min to 60 min and then the total sludge volume was centrifuged at 1400 x g for 10 min at 4°C. The sludge supernatant and sludge solids generated were separately subjected to the virus recovery methodology.

[c]The "overall virus recovery (%)" values were determined from the viruses recovered in the final concentrates and were based on the amount of viruses (total PFU) added to the sludge as 100%.

[d]Mean values displaying different superscript capital letters are significantly different at the 0.01 level when subjected to analysis of variance using Duncan's test.

[e]Abbreviation for standard error.

[f]The separated supernatants of the sludges with an asterisk were not processed for virus concentration. These supernatants were simply adjusted to neutral pH and assayed for viruses. The viruses recovered were included in the "overall virus recovery (%)" values displayed.

[g]The lagoon sludge is a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3), and consequently, its properties are close to those of anaerobically digested sludges (see Table 3-2). Therefore, lagoon sludges were placed in the population of anaerobically digested sludges.

digested sludges. The reason for the reduced recovery of viruses is that the elution step (i.e., mixing the sludge solids with five volumes of 0.05 M glycine buffer, pH 10.5 to 11.0, followed by rapid mixing for 30 seconds) is not effective for this sludge type. For example, the mean elution of solids-associated viruses for this sludge type was only 24%. In contrast, the mean elution of solids-associated viruses from mixed liquors and anaerobically digested sludges was 76% and 80%, respectively. The elution of viruses that was measured for mixed liquor solids (i.e., 76%), closely approaches the value of 84% reported by Hurst et al. (1978) for the elution of poliovirus type 1 from activated sludge solids. The reason for the poor elution of solids-associated viruses from aerobic sludges has not been determined. However, this sludge type was able to bind a larger fraction of poliovirus than mixed liquors or anaerobically digested sludges (see Table 3-4). In order to understand the mechanism(s) involved, the chemical and physical nature of the solids of different sludge types should be studied. All other virus adsorption-elution steps of the glycine method (i.e., virus concentration steps) were equally effective in poliovirus recovery for all sludge types tested.

Several methods have been proposed for the recovery of sludge solids-associated viruses and these methods are summarized in Table 3-8. Of the methods evaluated with enteroviruses seeded in sludge, all were tested for virus recovery efficiency using only one sludge type (see Table 3-8). The research presented above shows that sludge type influences the recovery of poliovirus from sludge solids. Although the

TABLE 3-8. Summary of the methods developed for the recovery of viruses from sludges

Eluent[a] used	Concentration technique for virus in eluate	Evaluation of the method for virus recovery		Reference
		Virus type(s) used	Sludge[b] type(s) used	
Sodium lauryl sulfate (0.1%) in 0.05 M glycine, pH 7.5	Organic flocculation	Poliovirus 1 (Sabin) Echovirus 7	Anaerobically digested (14 days)	Abid et al. (1978)
Beef extract (3%), ambient pH	Organic flocculation	Poliovirus 1 (CHAT)	Anaerobically digested Dewatered, composted	Glass et al. (1978)
Glycine buffer (0.05 M), pH 11.0	Organic flocculation	Poliovirus 1 (LSc) Coxsackievirus B3 (Nancy) Echovirus 7 (Wallace) Indigenous	Activated[c] Returned Aerobically digested (thickened, dewatered) Dried sludge (aerobically digested) from a sludge disposal site	Hurst et al. (1978)
Tryptose phosphate broth	None	Indigenous	Raw (primary) Anaerobically digested (40 days) Anaerobically digested (100 days)	Moore et al. (1978)
Glycine buffer, pH 11.0, beef extract or distilled water	Adsorption to bentonite clay	Indigenous	Raw (primary Anaerobically digested	Turk et al. (1980)
Beef extract (10%), pH 7.0, or Tris buffer, pH 9.0	None	Indigenous	Raw (primary and secondary)	Nielsen and Lydholm (1980)

			Anaerobically digested (30 days) Anaerobically digested (22-25 days) Dried sludge (anaerobically digested) sampled at the soil surface 1 day to 4 months after land application	
Beef extract (3%) or fetal calf serum (10%) both in saline, pH 7.2	None	Indigenous	Raw	Sattar and Westwood (1976)
Fetal calf serum (10%) in saline, pH 7.2	None	Indigenous	Raw Anaerobically digested (20 days) Lagoon-dried (anaerobically digested)	Sattar and Westwood (1979)
Fetal calf serum (2%) in Earle's balanced salt solution, pH 9.5	None	Poliovirus 1 (Sabin)	Digested	Subrahmanyan (1977)
Beef extract (3%), pH 9.0	Hydroextraction	Indigenous	Digested (40 days) Digested (> 60 days) Dried sludge cake from a sludge spray site	Wellings et al. (1976a)

[a]The chemicals listed were used to elute viruses from sludge solids.

[b]For some digested sludges, the type of digestion (i.e., aerobic or anaerobic) and/or the digestion time were not given in the literature.

[c]The effectiveness of this method in recovering seeded viruses was evaluated using activated sludge only. The method was subsequently used for recovering indigenous viruses from all the sludge types listed.

results pertain only to poliovirus type 1 (LSc) and to the glycine method used to recover the solids-associated viruses, the indication is that sludge type is an important factor that should be considered when assessing the effectiveness of virus recovery methods. Research conducted using sludge artificially contaminated with virus has been questioned because it is believed that, unlike indigenous viruses, the seeded viruses become mostly adsorbed to the surface of sludge solids (Moore et al. 1977; Nielsen and Lydholm 1980). Indigenous viruses are believed to be mostly embedded within the sludge solids rather than merely surface adsorbed (Wellings et al. 1976). In the case of wastewater-suspended solids, however, Stagg et al. (1978) demonstrated that most (85%) of the indigenous, solids-associated coliphages were adsorbed to the surface of sewage solids rather than embedded. The exact nature of the association between seeded or indigenous viruses and sludge solids has not yet been conclusively determined. Whereas research performed with virus-seeded sludge may not completely simulate natural conditions, valuable information can, nevertheless, be obtained in less time and at lower cost than when working with indigenous viruses. Using seeded viruses, the research reported herein has elucidated the role of sludge type in the recovery of viruses from sludge solids. The results of this study lead one to suggest that future methods developed for the recovery of viruses from sludges be evaluated for the various sludge types likely to be tested. There is clearly a need for a method that can be shown to be effective in the recovery of viruses from a variety of sludge types.

CHAPTER IV
POLIOVIRUS TRANSPORT STUDIES INVOLVING SOIL CORES TREATED WITH VIRUS-SEEDED SLUDGE UNDER LABORATORY CONDITIONS

Introduction

The application of wastewater sludge to land is receiving increased attention and will probably be the predominant sludge disposal method of the future (U.S. Environmental Protection Agency 1974, 1978b). There is some concern, however, that this practice may result in the contamination of groundwater supplies with pathogenic viruses (see reviews by Berg 1973b; Bitton 1975, 1980a; Bitton et al. 1979b; Burge and Marsh 1978; Burge and Parr 1980; Cliver 1976; Duboise et al. 1979; Elliott and Ellis 1977; Foster and Engelbrecht 1973; Gerba et al. 1975; Moore et al. 1978; Sagik 1975). Unfortunately, the transport pattern (i.e., movement or retention) of sludge-associated viruses in soils has not been adequately evaluated. The few studies that have been conducted indicate that enteroviruses seeded in anaerobically digested sludge are effectively retained by the soil matrix (Damgaard-Larsen et al. 1977; Moore et al. 1978; Sagik 1975). Since both seeded and indigenous viruses have been found to be associated with the sludge solids (Abid et al. 1978; Glass et al. 1978; Hurst et al. 1978; Lund 1971; Ward and Ashley 1976; Wellings et al. 1976a; also see Table 3-4), it follows that viruses are probably immobilized along with the sludge solids in the top portion of the soil profile (i.e., during surface spreading of sludge) or at the injection site within the soil

matrix (i.e., during subsurface injection of sludge) (Cliver 1976). Moreover, it appears that viruses are not readily dissociated from sludge solids in the soil environment (Burge and Parr 1980; Sagik 1975).

In this chapter, results of poliovirus (type 1, strain LSc) transport studies involving soil cores treated with virus-seeded sludge are presented. These studies were conducted under controlled laboratory conditions. The effect of sludge type (used anaerobically digested sludge, conditioned-dewatered sludge, chemical sludge and lime-stabilized, chemical sludge), soil type (used a Red Bay sandy loam and a Eustis fine sand), soil core type (used laboratory-packed soil columns and undisturbed soil cores) and application regime (virus-seeded sludge was applied continuously or in a spiked fashion) on the transport of sludge-associated poliovirus in soil cores was evaluated. The capacity of rain water to elute poliovirus from the sludge-soil matrix was also investigated. All soil cores were leached under saturated flow conditions. The information gained from this study should shed further light on the role of mineral soils in retaining sludge-associated viruses.

Materials and Methods

Virus and Viral Assays

Poliovirus type 1 (strain LSc) was used in the research reported in this chapter. Some general properties of polioviruses are shown in Table 3-1. Stocks of the virus were prepared as described in Chapter III (see page 53). The virus was kept at -70°C until used. Poliovirus was assayed by the plaque technique as described in Chapter III (see pages 53-56). Each viral count shown represents the average of

triplicate counts. The numbers of viruses were expressed as plaque-forming units (PFU).

Primary Wastewater Effluent

Primary wastewater effluent was obtained from the University of Florida campus wastewater treatment plant, Gainesville, Florida. The detention time of raw wastewater in the primary settlers was approximately 2 hours. No chlorine residual was found in the primary effluent sample used (i.e., by the orthotolidine test). The sample of primary effluent used was collected, and its pH and conductivity were measured as described below for digested sludges.

Sludges

Several sludge types were used in the research reported in this chapter and they are described below.

Digested sludges. Anaerobically digested sludges sampled at the Main Street wastewater treatment plants of Gainesville and Pensacola, Florida (GDAN and PDAN, respectively--see Table 3-2), were used. The sludges were collected and sludge parameters (i.e., pH and solids content) were measured as described in Chapter III (see page 56). The sludge conductivity was measured using a Beckman conductivity bridge model RC 16B2 (Beckman Instruments, Fullerton, California). Due to uncontrollable contamination of cell cultures during viral assays, these sludge samples had to be sterilized by autoclaving at 121°C with applied pressure of 15 psi for 15 minutes prior to use. As explained earlier (see page 59), autoclaving did not significantly affect the sludge-virus

interactions and the autoclaved sludge can simply be considered to be heat-conditioned. The sludges were used undiluted or diluted (1:50, vol./vol.) with either distilled water or 0.01 N calcium chloride. The pH and conductivity of each diluted sludge sample was also measured as described above for undiluted sludge.

Sludge liquor. Anaerobically digested sludge liquor was produced by centrifuging (all centrifugation was performed using a Sorvall RC5-B centrifuge, Ivan Sorvall Inc., Norwalk, Connecticut) GDAN sludge (see above) at 14,000 x g for 10 minutes at 4°C. This procedure was performed again on the decanted supernatant and this yielded the clear sludge liquor. Chemical parameters for this sludge liquor were determined by the Analytical Research Laboratory, Soil Science Department, University of Florida, Gainesville, and are presented in Table 4-1. The sludge liquor (containing 0.01 N calcium chloride) was also used to dilute (1:50, vol./vol.) the GDAN sludge. Lagoon sludge (LAG--see Table 3-2; a mixture of 1/3 aerobically digested sludge and 2/3 anaerobically digested sludge) liquor was also used and it was produced by the centrifugation of lagoon sludge as outlined above for GDAN sludge. The lagoon sludge liquor was passed through a series of 0.45- and 0.25-μm Filterite filters (Filterite Corp., Timonium, Maryland) in a 47-mm holder and then adjusted to pH 8.0 using 0.01 N NaOH prior to use. The pH and conductivity of each sludge liquor sample was measured as described above for undiluted digested sludges.

Conditioned-dewatered sludge. Poliovirus-seeded, GDAN sludge (see above) was conditioned with 1200 mg/ℓ of the cationic polymer,

TABLE 4-1. Chemical parameters for the anaerobically digested sludge liquor

Parameter[a]	Sludge[b] liquor value (ppm)	Parameter[a]	Sludge[b] liquor value (ppm)
Soluble salts	703	As	0
Na	63	Cd	0
K	29	Cr	0
Ca	20	Cu	0.05
Mg	15	Ni	0
Al	0	Pb	0
Fe	1.05	Zn	0.04

[a]Chemical parameters were determined by the Analytical Research Laboratory, Soil Science Department, University of Florida, Gainesville.

[b]Anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3250 μmho/cm at 25°C and 8.3, respectively) was centrifuged at 14,000 x g for 10 min at 4°C. This procedure was performed again on the decanted supernatant and this yielded a clear sludge liquor.

Hercufloc #871 (Hercules Co., Atlanta, Georgia). The polymer was added to 1000 ml of the virus-seeded sludge while mixing rapidly on a magnetic stirrer. Mixing was continued slowly for an additional 5 minutes. The entire sludge sample was then centrifuged at 320 x g (i.e., 1400 rpm) for 10 minutes at 25°C. The supernatant was decanted, assayed for viruses, and discarded. The dewatered sludge volume and sludge solids content (i.e., as percent, by method described above for digested sludges) was measured. The conditioned-dewatered sludge produced (i.e., in duplicate) was then assayed for viruses as described below. The procedure employed above in the conditioning and dewatering of the sludge was identical to that used at the Main Street wastewater treatment plant, Gainesville, Florida (Dr. DuBose, Main Street plant, personal communication; see Figure 6-1).

Chemical sludges. The chemical sludges were precipitated from 1000 ml of poliovirus-seeded, raw sewage using the general procedure of Sattar et al. (1976) and the coagulants, alum (i.e., aluminum sulfate), ferric chloride or lime (i.e., calcium hydroxide). The raw sewage used was obtained from the University of Florida campus wastewater treatment plant or from the Main Street wastewater treatment plant, both being located in Gainesville, Florida. The raw sewage samples were collected and sewage parameters (i.e., pH and conductivity) were measured as described above for sludge samples. The raw sewage samples were sterilized by autoclaving at 121°C with applied pressure of 15 psi for 15 minutes prior to use. Viral assays were made before and after the addition of coagulant. The final concentration in sewage of alum was 300 mg/ℓ

[as $Al_2(SO_4)_3 \cdot 18\ H_2O$] and of ferric chloride was 50 mg/ℓ (as $FeCl_3$). Similar concentrations of these coagulants were used by Wolf et al. (1974) in the precipitation of an activated sludge effluent as a tertiary treatment process. Following the addition of alum or ferric chloride, the pH of the solution was adjusted using 0.2 N HCl to 6.0 or 5.0, respectively, in order to achieve maximum flocculation (Fair et al. 1968). The coagulant, lime, was added until a pH of 11.1 to 11.3 [i.e., final concentration in sewage of 150 to 250 mg/ℓ of $Ca(OH)_2$] was achieved (according to Sattar et al. 1976). Following the addition of the coagulants, the sewage samples were mixed on a magnetic stirrer rapidly for 10 minutes and slowly for 5 minutes. The flocculated sewage samples were then transferred to Imhoff cones and 60 minutes was allowed for the formation and settling of the chemical sludges (see Figure 4-1). The supernatants in the Imhoff cones were assayed for viruses and discarded. The sludge volume and sludge solids content (i.e., as percent, by method described above for digested sludges) was measured for each chemical sludge. The chemical sludges produced (i.e., in duplicate for the lime sludge, and in triplicate for alum and ferric chloride sludges) were then assayed for viruses as described below.

Lime-stabilized, chemical sludges. A sample of alum sludge and a sample of ferric chloride sludge, produced and assayed for viruses as described above, were lime-stabilized according to the procedure of Farrell et al. (1974). The sludges were treated with an aqueous slurry of lime [5% (wt./vol.) stock of $Ca(OH)_2$] until a pH of 11.5 was achieved and maintained for 5 minutes. The final concentration of lime [as

FIGURE 4-1. Chemical sludges (i.e., lime and alum) were precipitated from poliovirus-seeded, raw sewage and are shown after settling for 60 minutes in Imhoff cones


Lime
sludge
Alum
sludge

$Ca(OH)_2$] added to the alum and ferric chloride sludge was 1389 and 625 mg/ℓ, respectively. A contact time of 30 minutes was allowed while mixing the suspension on a magnetic stirrer. After 30 minutes of mixing, the pHs of the sludges were measured and had dropped to 11.1 or 11.3. The lime-stabilized, chemical sludges were then assayed for viruses as described below.

Association of Seeded Poliovirus with Sludge Solids

Poliovirus stock in phosphate-buffered saline (PBS) containing 2% fetal calf serum (FCS) (see Appendix for more details on the composition of this solution) was added directly to 0.01 N calcium chloride, primary wastewater effluent, anaerobically digested sludge (diluted or undiluted) and sludge liquor at the rate of 1 ml of virus stock per 1000 ml of solution and while stirring the suspension using a magnetic stirrer (see Chapter III, page 59). After a 1-minute mixing period, an aliquot of the samples containing low solids contents (i.e., 0.01 N calcium chloride, primary wastewater effluent, diluted anaerobically digested sludge, and sludge liquor) was diluted in PBS containing 2% FCS and assayed directly for seeded viruses by the plaque technique. This viral assay was performed in order to determine the amount of virus present in these samples initially and was repeated at the end of experimental trials in order to assess any viral inactivation which might have occurred. No attempt was made to determine the degree of association between seeded poliovirus and the small quantity of solids present in these samples.

The association of poliovirus with sludge solids was determined for undiluted anaerobically digested sludge (seeded with

poliovirus and suspension magnetically mixed for 10 to 60 minutes), conditioned-dewatered sludge (poliovirus transferred to this sludge during dewatering process), chemical sludges (poliovirus transferred to chemical sludges during precipitation of virus-seeded, raw sewage) and lime-stabilized, chemical sludges. The procedures used are outlined in detail in Chapter III (see page 62). Briefly, an aliquot of the unfractionated sludge (i.e., sludge sample without solids separated) was diluted in PBS containing 2% FCS and assayed directly for viruses by the plaque technique. This method (i.e., sludge dilution and subsequent direct assay on cell cultures) has been previously shown to be highly efficient in the recovery of poliovirus from unfractionated sludge (see Chapter III, page 62 and Table 3-3). The unfractionated sludge assay was performed in order to determine the total amount of virus present in the sludge sample. An aliquot of the sludge was subsequently centrifuged at 1400 x g for 10 minutes at 4°C. The sludge supernatant produced was assayed for viruses. The "viable unadsorbed virus" and "sludge solids-associated virus" fractions were calculated as shown in Chapter III (see page 64).

Rain Water

Rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. Chemical parameters for the rain water used were determined by Hendry (1977) and are presented in Table 4-2. The rain water was sterilized by autoclaving at 121°C with applied pressure of 15 psi for 15 minutes prior to use.

TABLE 4-2. Chemical parameters for the rain water used in this study

Parameter	Value[a]	Parameter	Value[a]
pH	4.46	K	0.15
Conductance[b]	23.9	Ca	0.50
TOC	5.20	Mg	0.07
TKN	0.69	Cl^-	0.71
NH_4^+-N	0.10	SO_4^{-2}	1.84
NO_3^--N	0.17	Cd	5.7
Ortho-P	0.016	Pb	15.2
Total-P	0.032	Cu	39.4
Na	0.33	Zn	28.2

[a]Data were adapted from Hendry (1977). The values shown represent average weighted concentration of individual rain events collected next to the Environmental Engineering Sciences building, University of Florida, Gainesville, from June 1976 to May 1977.

[b]Value in μmho/cm. The values for Cd, Pb, Cu, and Zn are in μg/l. All other values except pH are in mg/l.

Soils

The soils studied were a Red Bay sandy loam sampled at the West Florida Agricultural Experiment Station, Jay, and a Eustis fine sand sampled at the agronomy farm, University of Florida, Gainesville. The Red Bay sandy loam has been classified as a Rhodic Paleudult, fine-loamy, siliceous, thermic while the Eustis fine sand was classified as a Psammentic Paleudult, sandy, siliceous, hyperthermic (Calhoun et al. 1974). Some characteristics of these soils are shown in Table 4-3. The percent organic matter in these two soils was measured at less than 1% except for the A1 horizon of the Red Bay sandy loam which was found to contain 4.3% organic matter (Calhoun et al. 1974).

Poliovirus Transport Studies

Poliovirus transport (i.e., movement or retention) in soil cores treated with virus-seeded sludge was studied under laboratory conditions. Two types of soil cores were used as described below.

Laboratory-packed soil columns. Laboratory-packed soil columns were prepared using subsoil samples of Red Bay sandy loam (consisted mainly of the A2 and B1t horizons--see Table 4-3) and Eustis fine sand (consisted mainly of the A21 and A22 horizons--see Table 4-3). Each subsoil sample was screened by hand to remove rocks and large organic matter, and was then allowed to air dry. The soils were not autoclaved or sterilized in any other way. The dry soils were then carefully packed into acrylic plastic columns 10 cm or 29 cm in length (packed 10 cm or 27 cm with soil, respectively) and 4.8-cm internal diameter. A polypropylene screen (105-μm pore size) was used to support the soil

TABLE 4-3. Some characteristics of the soils under study

Soil[a]	Soil horizon	Depth (cm)	Description	Mechanical composition (%)		
				Sand (2-0.05 mm)	Silt (0.05-0.002 mm)	Clay (< 0.002 mm)
Red Bay sandy loam[c]	A1	0-15	Dark brown fine sandy loam	66.0	20.4	13.6
	A2	15-30	Yellowish-red sandy loam	61.0	20.0	19.0
	B1t	30-48	Red sandy clay loam	56.0	15.4	28.6
	B21t	48-97	Red light sandy clay	52.4	11.4	36.2
Eustis fine sand[d]	Ap	0-25	Dark gray fine sand	94.8	2.4	2.8
	A21	25-58	Light yellowish-brown fine sand	94.4	2.0	3.6
	A22	58-102	Light yellowish-brown fine sand	94.3	2.3	3.4
	A23	102-135	Light yellowish-brown fine sand	94.7	1.6	3.7
	A24	135-163	Yellowish-brown fine sand	93.9	1.3	4.8

[a]Adapted from Calhoun et al. (1974).

[b]Identification by x-ray diffraction.

[c]Sample was taken in Santa Rosa County, Florida; West Florida Agricultural Experiment Station, Jay.

[d]Sample was taken in Alachua County, Florida; agronomy farm, University of Florida, Gainesville.

TABLE 4-3. Extended.

Dominant clay[b]	pH (in 1:1 water)	Bulk density (g/cm^3)	Saturated hydraulic conductivity (cm/hr)
Vermiculite	5.3	1.26	12.1
Vermiculite	5.4	1.43	15.9
Vermiculite	5.6	1.48	20.2
Gibbsite	5.1	1.60	21.6
--	6.7	1.62	28.0
--	6.5	1.59	27.2
--	6.5	1.54	34.0
--	6.3	1.55	52.6
--	6.0	1.53	54.2

in each column while allowing the free movement of viruses (i.e., did not adsorb viruses in soil leachates). The columns were packed uniformly at a constant rate while gently tapping to prevent soil subsidence (Drewry and Eliassen 1968). The field bulk densities of 1.45 g/cm^3 for the Red Bay sandy loam (i.e., average for the A2 and B1t horizons--see Table 4-3) and 1.56 g/cm^3 for the Eustis fine sand (i.e., average for the A21 and A22 horizons--see Table 4-3) were reproduced in the soil columns by packing the appropriate grams of dry soil in the measured volume of each column (i.e., 180.86 cm^3 for the 10-cm column and 488.33 cm^3 for the 27-cm column). The soil columns were then placed in soil column holders (supplied by Soil Moisture Equipment Corp., Santa Barbara, California) as shown in Figures 4-2 and 4-3 for 10-cm and 27-cm columns, respectively. The air in the columns was then displaced by flushing with carbon dioxide for approximately 60 minutes in order to ensure subsequent uniform wetting of the soil. The soil columns were then conditioned by passing 2 to 5 pore volumes of non-seeded 0.01 N calcium chloride, distilled water, rain water, or sludge liquor using a peristaltic pump (Buchler, Fort Lee, New Jersey). The conditioning solution used was identical or similar to the test solution. For example, soil columns ultimately receiving virus-seeded, anaerobically digested sludge diluted (1:50, vol./vol.) with 0.01 N calcium chloride were previously conditioned with 0.01 N calcium chloride. For soil columns receiving undiluted sludge (e.g., chemical sludge), rain water was used as the conditioning solution. Following conditioning, poliovirus was suspended in 0.01 N calcium chloride, sludge

FIGURE 4-2. Laboratory-packed soil columns (10 cm in length) used in poliovirus transport studies

The soil columns (4.8 cm internal diameter) were packed as described in the Materials and Methods section, and were then placed in soil column holders. The soil column holders were supplied by the Soil Moisture Equipment Corp. (Santa Barbara, California). Leaching solutions were applied to the soil columns using a peristaltic pump (Buchler, Fort Lee, N.J.) as shown.

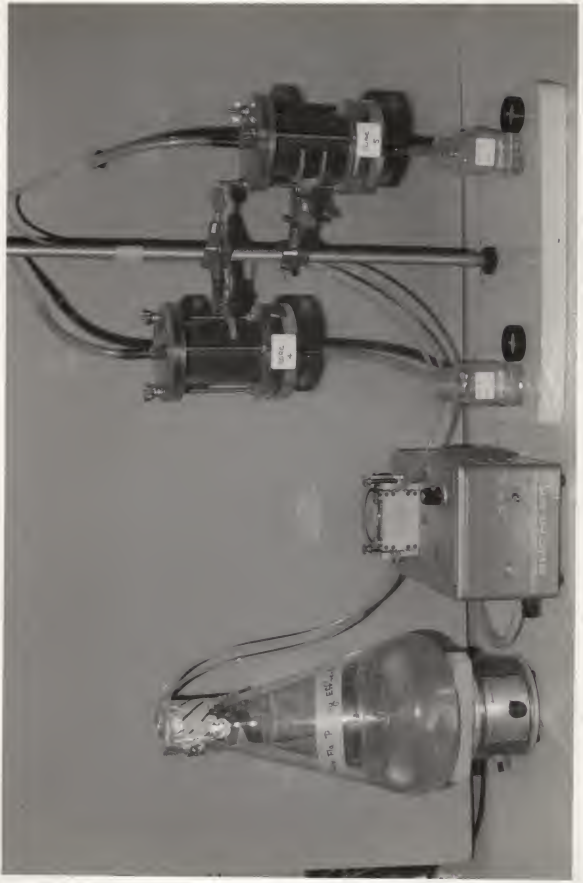
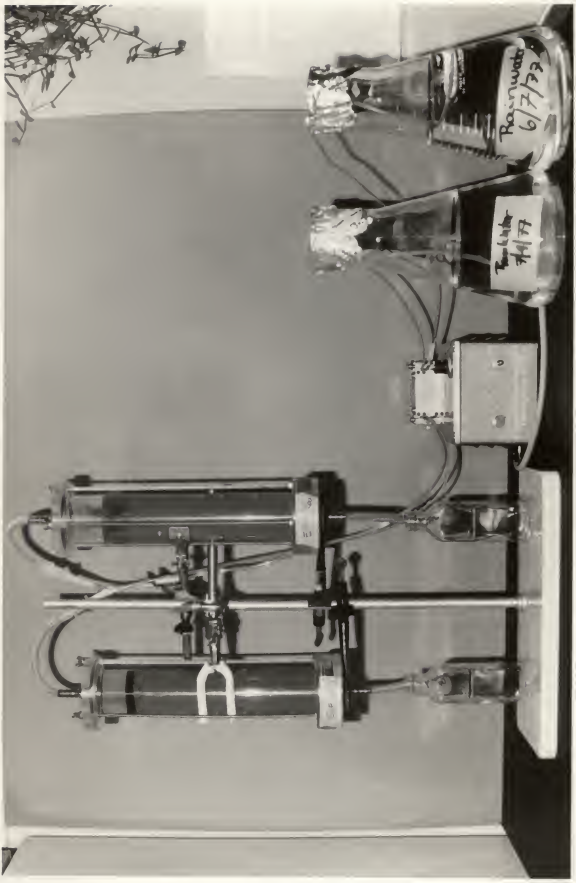


FIGURE 4-3. Laboratory-packed soil columns (27 cm in length) used in poliovirus transport studies

The soil columns (4.8 cm internal diameter) were packed as described in the Materials and Methods section, and were then placed in soil column holders. The soil column holders were supplied by the Soil Moisture Equipment Corp. (Santa Barbara, California). Leaching solutions were applied to the soil columns using a peristaltic pump (Buchler, Fort Lee, N.J.) as shown.


Rainwater
6/7/93
Tap water
4/4/77

liquor or diluted anaerobically digested sludge and was subsequently applied continuously to soil columns at approximately 5 ml/min using the peristaltic pump. As detailed above, influent poliovirus concentration was determined from viral assays made at the beginning and end of each column experiment. In several experiments, a shift was made to the application of nonseeded 0.01 N calcium chloride or rain water continuously (at approximately 5 ml/min) by the peristaltic pump in order to determine if these solutions could elute adsorbed viruses. The undiluted sludge samples (i.e., conditioned-dewatered sludge, chemical sludges and lime-stabilized, chemical sludges) could not be applied to the soil columns via the peristaltic pump because of the high solids contents. These sludges were applied directly on top of the soil columns, were allowed to soak in, and then, were worked under 2.5 cm. The poliovirus concentrations in these sludges was determined as described earlier. Following the application of the sludges, the covers on the soil columns (i.e., the top plates of the soil column holders) were replaced and the soil columns were then leached with nonseeded rain water. The rain water was applied continuously to the soil columns at approximately 5 ml/min using the peristaltic pump. After percolation through the soil, the leachates from all laboratory-packed columns were collected in sterile screw-capped bottles and assayed for viral infectivity as described below.

Undisturbed soil cores. Undisturbed soil cores (Blake 1965; Sanks et al. 1976) were also used in poliovirus transport studies. Undisturbed cores were obtained in driving polyvinyl chloride pipes

into the soil to the desired depth according to the procedure of Blake (1965). Care was taken not to compress the soil in the pipes during sampling, and, thereby, to preserve the natural structure and packing of the soil as nearly as possible in the pipes. This was accomplished by ensuring that the elevation of the soil inside the pipes was the same as the elevation of the surface soil outside the pipes during sampling (Blake 1965). The soil around the pipes was then removed with a shovel and the soil cores were gently removed. In the laboratory, soil extending beyond the bottom end of each core was trimmed with a spatula. A polypropylene screen (105-μm pore size--see above) and a spout were secured at the bottom of each undisturbed soil core. Red Bay sandy loam undisturbed cores were sampled at the West Florida Agricultural Experiment Station at Jay. These soil cores were 54 cm in length (pipes were 60 cm in length) and had an internal diameter of 5.0 cm, and, thereby, consisted of the A1, A2, B1t and B21t horizons of the sandy loam (see Table 4-3). The Red Bay sandy loam cores were not conditioned prior to use. Eustis fine sand undisturbed cores were sampled at the agronomy farm, University of Florida, Gainesville. These soil cores were 33 cm in length (pipes were 40 cm in length) and had an internal diameter of 5.0 cm, and thereby, consisted of the Ap and A21 horizons of the fine sand (see Table 4-3). The Eustis fine sand cores were conditioned with either 5 pore volumes of rain water or 0.01 N calcium chloride. All undisturbed soil cores were then treated with undiluted anaerobically digested sludge (GDAN--see above) which had been seeded with poliovirus. The poliovirus concentrations in the anaerobically

digested sludge samples and the degree of poliovirus association with the sludge solids were determined as described earlier in this chapter. Following viral assays, one inch or 2.5 cm (51.6 ml) of poliovirus-seeded sludge was applied to each soil core, allowed to soak in and then was worked under 2.5 cm. In one experiment with two Red Bay sandy loam cores, the applied sludge was allowed to air dry for 24 hours (the cores were placed on the roof of the Environmental Engineering Sciences building, University of Florida, Gainesville) before being worked under 2.5 cm. Following incorporation of the sludge solids into the top inch of soil, the soil cores were eluted with either 0.01 N calcium chloride or rain water. These solutions were applied from inverted, self-regulated, 1-liter Erlenmeyer flasks set to maintain a 2.5-cm hydraulic head on the cores (Sanks et al. 1976). After percolation through the soil, leachates from the undisturbed soil cores were collected in sterile screw-capped bottles and assayed for viral infectivity as described below. The percolation rate of fluid through each undisturbed soil core was determined by measuring the time required to collect a known volume of leachate.

Leachates from transport studies. The leachates from laboratory-packed soil columns and undisturbed soil cores were collected in pore volumes or fractions of pore volumes. The pore volume of each soil core (or column) is the volume within the core which is not occupied by the soil particles (Brady 1974) and is determined by first calculating the percent pore space of the soil as shown below:

$$\text{pore space } (\%) = 100 - \left(\frac{\text{bulk density}}{\text{particle density}} \times 100\right) \qquad (4\text{-}1)$$

The bulk density is defined as the mass of dry soil in a unit volume consisting of soil solids and pores (Blake 1965; Brady 1974). The particle density is defined as the mass of a unit volume of soil solids (Brady 1974). The bulk density of the Red Bay sandy loam was 1.45 g/cm^3 (used this value for both laboratory-packed columns and undisturbed cores), and that of the Eustis fine sand was 1.56 or 1.61 g/cm^3 (for laboratory-packed columns or undisturbed cores, respectively). An average value of 2.60 g/cm^3 (range from 2.52 to 2.69 g/cm^3) was used for particle density. These values of particle density are typical for mineral soils (Brady 1974). The pore volume of each core was then calculated as shown below:

$$\text{pore volume (ml)} = \frac{\text{pore space (\%)}}{100} \times \text{total volume (ml) of core} \qquad (4\text{-}2)$$

An aliquot of each leachate sample was diluted (i.e., if necessary) in PBS containing 2% FCS and assayed directly for viruses by the plaque technique. In order to detect small numbers of viruses, the leachates from laboratory-packed soil columns which had received chemical sludges and lime-stabilized, ferric chloride sludge were concentrated by membrane filtration (Farrah et al. 1976; Hill et al. 1971; Shuval and Katzenelson 1972; Sobsey et al. 1973; Sobsey et al. 1980b). These soil leachates were collected in 1/2 pore volume fractions which were assayed individually for viral infectivity as described above. Pore volumes 0.5 through 5.0 (and 5.5 through the

final pore volume) were subsequently combined and concentrated 160-fold as follows. The leachate sample was adjusted to pH 3.5 by the addition of 1 M glycine buffer, pH 2.0, and adjusted to a final concentration of 0.0005 M aluminum chloride. The treated sample was then passed through a series of 3.0- and 0.45-μm Filterite filters in a 47-mm holder. Adsorbed viruses were eluted from the filters with 7 ml of PBS containing 10% FCS, pH 9.0. The filter eluate was adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for viruses by the plaque technique.

The quantity of poliovirus detected in each soil leachate was expressed as a percentage of the amount of virus applied to the soil. For cores receiving poliovirus continuously (i.e., laboratory-packed soil columns), the quantity of poliovirus leached was expressed as the cumulative percent of the total viral PFU having been applied at each pore volume and it was calculated by the following equation:

$$\begin{array}{l}\text{poliovirus eluted at pore volume b (as cumulative \% of total PFU having been applied at pore volume b)}\end{array} = \frac{\left[\frac{\text{pore volume (ml)}}{a}\right] \times \left[\sum_{pv=1/a}^{pv=b} \text{poliovirus eluted in each 1/a pore volume (PFU/ml)}\right]}{\left[b \times \text{pore volume (ml)}\right] \times \left[\text{influent poliovirus concentration (PFU/ml)}\right]} \times 100 \qquad (4\text{-}3)$$

where pv represents the pore volume number; the leachate samples were collected and assayed in 1/a pore volume fractions (a set at 1, 2, or 3

in the research reported herein); and b can be set at any value between 1/a and the final pore volume number collected. For cores receiving poliovirus in a spiked fashion (i.e., all at once at the beginning of the experimental trial; used this procedure in both laboratory-packed soil columns and undisturbed soil cores), the quantity of poliovirus leached was expressed as the cumulative percent of the total viral PFU applied and it was calculated by the following equation:

$$\begin{array}{l}\text{poliovirus eluted at pore volume b (as cumulative \% of total PFU applied)} \\ = \dfrac{\left[\dfrac{\text{pore volume (ml)}}{a}\right] \times \left[\sum_{pv=1/a}^{pv=b} \text{poliovirus eluted in each 1/a pore volume (PFU/ml)}\right]}{\text{poliovirus applied (total PFU)}} \\ \qquad \times 100 \end{array} \qquad (4\text{-}4)$$

where pv, a, and b are defined as in Equation (4-3) above.

The pH and conductivity of each soil core leachate were measured as described above for undiluted digested sludges.

Distribution of virus in the soil. The distribution of poliovirus in the soil profile was studied in two 27-cm laboratory-packed columns of Eustis fine sand. These soil columns were prepared and treated with virus-seeded, diluted (1:50 with 0.01 N calcium chloride or distilled water) anaerobically digested sludge as described above. The leachates from these columns were collected and assayed for viruses as described earlier. Following leaching, the soil profile in

each column was separated into 2- to 3-cm sections. The sludge solids resting on top of the soil were also separated and considered as one section. Each soil (or sludge solids) section was well mixed in a sterile beaker with a spatula, wet weighed, and then a representative sample (10 grams of wet soil; total known amount of top wet sludge solids) was taken and subjected to the following virus recovery methodology. Each sample was mixed with 3% (wt./vol.) beef extract (Difco Laboratories, Detroit, Michigan), buffered with Tris(hydroxymethyl)-aminomethane (Sigma Chemical Co., St. Louis, Missouri) at pH 9.0 in the proportion of 1 gram of wet soil (or wet sludge solids) per 2 ml of eluent. This solution has been found to be effective in the elution of poliovirus type 1 from soil (Bitton et al. 1979a) and from sludge solids (Farrah et al. 1981b). The mixtures were then vortexed for 30 seconds and sonicated for 3 minutes at maximum deflection (60 watts) using a Branson sonifier (Branson Instruments Inc., Danbury, Connecticut). The samples were then centrifuged at 1900 x g for 10 minutes at 4°C. The supernatants were adjusted to neutral pH. An aliquot of each supernatant produced was then diluted (i.e., if necessary) in PBS containing 2% FCS and assayed directly for viruses by the plaque technique. The amount of poliovirus recovered was expressed as PFU per soil (or sludge solids) section.

Effect of Soil Bulk Density on Poliovirus Transport

The poliovirus transport studies described above were conducted with laboratory-packed soil columns and undisturbed soil cores displaying similar bulk densities as found in the field for the two soils studied

(see Table 4-3). In the laboratory-packed columns, the field bulk densities were accurately reproduced as detailed earlier. The undisturbed soil cores were obtained in a manner that preserved the natural structure and bulk density of the soil (see page 102 above). In fact, undisturbed soil cores are frequently taken in order to measure the field bulk density of a soil (Blake 1965). However, the soil in undisturbed cores can be compressed during sampling and thereby result in an increase in bulk density as compared to the field bulk density (Blake 1965; Funderburg et al. 1979). For example, compression of the soil is likely to occur when the soil is wet during sampling (Blake 1965). Although the undisturbed cores used in this study were carefully sampled so as to not compress the soil, it was deemed important to determine what effect, if any, compression of the soil (i.e., increase in bulk density) might have on the transport of poliovirus. Laboratory-packed soil columns of Red Bay sandy loam subsoil (consisted mainly of the A2 and B1t horizons--see Table 4-3) were used in these experiments and they were prepared at different bulk densities as described below.

Soil moisture content-bulk density curve. If the compactive force is held constant, the density to which a given soil can be compacted increases with a corresponding increase in the soil moisture content up to the optimum moisture level (Felt 1965). Increases in soil moisture content beyond this level result in reductions in the soil bulk densities achieved (Felt 1965). A soil moisture content-bulk density curve was produced for the Red Bay sandy subsoil using the procedure of Wilson (1950). Briefly, the procedure consisted of adjusting

air-dried soil to moisture contents ranging from 7.3% to 14.4% (wt./wt.) using rain water and then compacting each sample in the Harvard compaction apparatus (Soiltest Inc., Evanston, Illinois). Each soil sample was added to the compaction mold (known volume) in 3 layers with a compactive force of 10 tamps applied per layer (at different positions) using a 20-lb (9.1-kg) tamper (Soiltest Inc., Evanston, Illinois; see Wilson 1950). The soil sample was then ejected from the mold, wet weighed, and dried to constant weight. The exact soil moisture content and dry compacted bulk density were calculated for each sample. The soil moisture content-bulk density curve obtained for the Red Bay sandy loam subsoil is shown in Figure 4-4. For the compactive force used, a maximum bulk density of 1.96 g/cm^3 was achieved when the soil moisture content was 12.4% (i.e., optimum moisture content). It should be noted that cohesionless soils (i.e., sands) are compacted to maximum density by simply vibrating the air-dried soil (Felt 1965). However, the increase in bulk density achieved by maximum compaction is much smaller for sands than for finer textured soils (Brady 1974; Freeze and Cherry 1979). This phenomenon was observed with the Eustis fine sand subsoil (consisted mainly of the A21 and A22 horizons--see Table 4-3) for which the maximum bulk density attained was only 1.70 g/cm^3. Thus, the Red Bay sandy loam subsoil was used here because of the greater range in bulk densities which could be produced with this soil.

Saturated hydraulic conductivity. Prior to initiating poliovirus transport studies, it was important to determine the effect compaction (i.e., increase in bulk density) of the Red Bay sandy loam

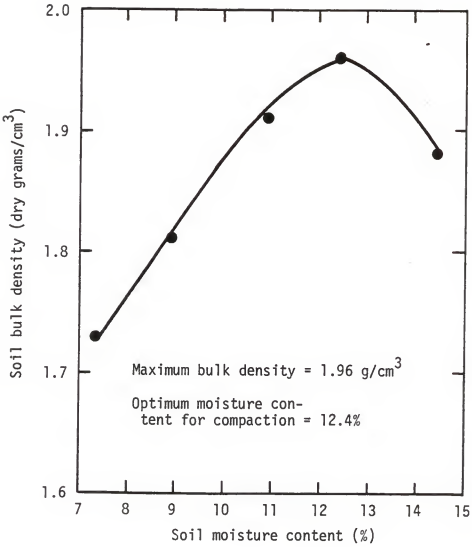


FIGURE 4-4. Soil moisture content-bulk density curve for the Red Bay sandy loam subsoil

Air-dried samples of Red Bay sandy loam subsoil (i.e., consisting of the A2 and B1t horizons--see Table 4-3) were adjusted to moisture contents ranging from 7.3% to 14.4% (wt./wt.). The soil samples were then compacted using the procedure of Wilson (1950).

subsoil would have on the rate of water movement through the soil. The soil was packed into acrylic plastic columns (10 cm in length and 4.8-cm internal diameter; packed 5.0 cm with soil) at bulk densities of 1.45, 1.60, 1.70, 1.85, and 2.00 dry g/cm^3. A polypropylene screen (105-μm pore size) was used to support the soil in each column. The columns at bulk densities of 1.45 and 1.60 g/cm^3 were packed with air-dried soil while tapping on the outside of the columns. The amount of tapping was increased to achieve the higher bulk density (i.e., 1.60 g/cm^3). The columns at bulk densities of 1.70, 1.85, and 2.00 g/cm^3 were packed with moist soil [12.4% (wt./wt.) moisure content was adjusted with rain water]. As shown above, 12.4% is the optimum moisture content for the compaction of this soil. The moist soil was compacted in the soil columns in 3 layers with 13, 20, or 33 tamps applied per layer [using a 20-lb (9.1-kg) tamper as described by Wilson (1950)--see above] in order to obtain a bulk density of 1.70, 1.85, or 2.00 g/cm^3, respectively. The saturated hydraulic conductivity of each soil column was then measured using the "constant-head" method of Klute (1965). Briefly, the procedure consisted of applying a constant, hydraulic head of 2.5 cm to each saturated soil column using rain water and then measuring the volume of leachate collected in a measured time. When possible, the leachate sample was collected within 30 minutes of the beginning of leaching as recommended by Klute (1965). The saturated hydraulic conductivity was calculated by the following equation (Klute 1965):

$$K = (Q/At)(L/\Delta H) \qquad (4\text{-}5)$$

where

K = saturated hydraulic conductivity (in cm/hr)

Q = volume of leachate (in cm^3)

A = cross-sectional area of soil sample (18.09 cm^2 for the soil columns in this study)

t = time to collect volume of leachate (in hr)

L = length of soil sample (5.0 cm)

ΔH = hydraulic head difference (7.5 cm)

From Table 4-4, it is clear that the saturated hydraulic conductivity of the Red Bay sandy loam subsoil decreased as the bulk density of the soil increased from 1.45 to 2.00 g/cm^3. At bulk densities of 1.70 g/cm^3 or greater, the saturated hydraulic conductivities were drastically reduced (see Table 4-4). Therefore, soil columns packed a these high bulk densities (i.e., $\geq$1.70 g/cm^3) could not be used in subsequent poliovirus transport experiments because of the large amount of time required to collect an adequate volume of leachate.

Poliovirus transport. The effect of soil bulk density on poliovirus transport was studied using laboratory-packed soil columns of Red Bay sandy loam subsoil. The air-dried soil (not autoclaved) was packed into acrylic plastic columns (10 cm in length and 4.8-cm internal diameter; packed 10 cm with soil) at bulk densities of 1.45 and 1.60 g/cm^3 while tapping on the outside of the columns. The characteristics of the soil columns packed at these bulk densities are shown in Table 4-5. As the bulk density of the soil was increased from 1.45 to 1.60 g/cm^3, there was a corresponding decrease in the percent pore

TABLE 4-4. Effect of soil bulk density on the saturated hydraulic conductivity of the Red Bay sandy loam subsoil

Bulk density[a] (dry g/cm^3)	Saturated hydraulic conductivity[b]		Permeability class[d]
	ml/min	cm/hr[c]	
1.45	33	73	Very rapid
1.60	8.2	18	Rapid
1.70	0.35	0.77	Moderately slow
1.85	0[e]	0	Very slow
2.00	0[e]	0	Very slow

[a]The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The bulk densities shown were produced in soil columns (i.e., 10 cm in length and 4.8-cm internal diameter; packed 5.0 cm with soil) as described in the Materials and Methods section.

[b]The saturated hydraulic conductivity of each soil column was measured using the "constant-head" method of Klute (1965).

[c]Calculated using Equation (4-5).

[d]According to Klute (1965).

[e]No leachate passed through these columns in a 2-hour period.

TABLE 4-5. Characteristics of the 10-cm columns of Red Bay sandy loam subsoil packed at bulk densities of 1.45 and 1.60 g/cm^3

Bulk density[a] (g/cm^3)	Particle density[b] (g/cm^3)	Pore space[c] (%)	Pore volume[d] (ml)
1.45	2.60	44.2	79.9
1.60	2.60	38.5	69.6

[a]The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The bulk densities shown were produced in the soil columns (i.e., 10 cm in length and 4.8-cm internal diameter) by packing the appropriate grams of air-dried soil in the column volume.

[b]This particle density value is typical for mineral soils (Brady 1974).

[c]Calculated using Equation (4-1).

[d]Calculated using Equation (4-2).

space and pore volume of the 10-cm soil column (see Table 4-5). Three columns were prepared at each bulk density. A polypropylene screen (105-μm pore size) was used to support the soil in each column. The soil columns were placed in soil column holders and treated with carbon dioxide as described earlier (see page 97). The soil columns were then conditioned by passing 2 pore volumes of nonseeded primary wastewater effluent using the peristaltic pump. Following conditioning, poliovirus was suspended in primary wastewater effluent (as described on page 91) and subsequently applied continuously to the soil columns at approximately 3.5 ml/min using the peristaltic pump. As detailed earlier (see page 91), influent poliovirus concentration was determined from viral assays made at the beginning and end of each column experiment. After percolation through the soil, the column leachates were collected in pore volumes using sterile screw-capped bottles. The poliovirus content, pH and conductivity of the leachates were determined as described earlier (see pages 104-107).

Statistical treatment of data. Statistical treatment of the data was performed with the use of a Hewlett-Packard calculator model 9810A and Statistics Package V-6 (Hewlett-Packard Company, Loveland, Colorado).

Results and Discussion

Prior to conducting field experiments, it appeared necessary to study the transport pattern (i.e., movement or retention) of sludge-associated viruses in the soils under consideration, Eustis fine sand and Red Bay sandy loam. Experiments were undertaken to study viral

transport under optimal conditions (i.e., in the presence of 0.01 N $CaCl_2$) and under more realistic conditions involving sludge application to soils.

Poliovirus Suspended in 0.01 N $CaCl_2$

Red Bay sandy loam. The "retention potential" of this soil towards poliovirus was first determined, under optimal conditions, in the presence of 0.01 N $CaCl_2$. As shown in Table 4-6, more than 99.99% of the viral load was removed, presumably due to adsorption, after 10 pore volumes of solution had passed through the soil. The pH of the soil solution varied from 5.1 to 6.3 and the conductivity was around 1300 μmhos/cm (see Table 4-6). A shift from calcium chloride to rain water did not result in any appreciable release of soil-bound viruses although the conductivity of the soil solution decreased from 1320 to 54 μmhos/cm. It is well known that divalent cations, at appropriate concentrations, enhance the adsorption of viruses to soils (Bitton 1975; Drewry and Eliassen 1968; Gerba et al. 1975; Lefler and Kott 1974). Rain water may be important in the redistribution and transport of viruses through the soil matrix. This has been suggested in the field (Wellings et al. 1975) and demonstrated in the laboratory (Duboise et al. 1976; Lance et al. 1976). However, it was found that rain water did not significantly affect the desorption of viruses from a soil containing 28% clay (Scheuerman et al. 1979).

Eustis fine sand. The "retention potential" of this soil towards poliovirus was also evaluated, as described above for the Red Bay sandy loam, in the presence of 0.01 N $CaCl_2$. It was observed

TABLE 4-6. Retention of poliovirus type 1 by a packed column of Red Bay sandy loam subsoil when suspended in 0.01 N $CaCl_2$ and after subsequent application of rain water

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	% of Total PFU having been applied at each pore volume (cumulative)	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
			0.01 N $CaCl_2$[c] seeded with poliovirus		
1	0	0	0	1260	6.3
2	0	0	0	1300	6.1
3	11	0.046	0.02	1340	5.4
4	0	0	0.01	1380	6.3
5	0	0	0.009	1320	5.2
6	0	0	0.008	1320	5.4
7	0	0	0.007	1360	5.1
8	0	0	0.006	1340	5.1
9	0	0	0.005	1320	5.1
10	0	0	0.004	1350	5.2
			Shift to nonseeded rain water[d]		
11	2	0.008	0.005	1320	6.4
12	0	0	0.005	740	5.3
13	0	0	0.005	168	4.8
14	0	0	0.005	73	6.0

15	0	0	0.005	62	5.8
16	0	0	0.005	74	6.5
17	0	0	0.005	61	6.5
18	0	0	0.005	60	6.3
19	0	0	0.005	59	6.6
20	0	0	0.005	54	6.7

[a]One pore volume for the column used equals 80 ml. The laboratory-packed column was 10 cm in length and 4.8 cm internal diameter. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The column was conditioned with 5 pore volumes of 0.01 N $CaCl_2$. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.).

[b]Poliovirus was seeded in the influent (i.e., 0.01 N $CaCl_2$) at a concentration of 2.4×10^4 PFU/ml.

[c]The conductivity of 0.01 N $CaCl_2$ was 1210 μmho/cm at 25°C and the pH was 6.4.

[d]Rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.

(see Table 4-7) that the maximum breakthrough per pore volume was 0.3%. This table also shows that approximately 99.3% of the viral load was retained by this soil following leaching with 10 pore volumes of seeded 0.01 N $CaCl_2$. As in the case of the Red Bay sandy loam (see Table 4-6), this sandy soil has displayed a substantial capability of removing poliovirus suspended in a calcium chloride solution.

Poliovirus Suspended in Diluted Anaerobically Digested Sludge

Red Bay sandy loam. Anaerobically digested sludge (2% solids content, w/v), diluted 1 to 50 (v/v) with 0.01 N calcium chloride or distilled water, was seeded with poliovirus and thoroughly mixed to bring about the adsorption of the virus to the sludge particles. The virus-sludge mixtures were then pumped onto the top of Red Bay sandy loam columns. The dilution of the sludge (1:50) with calcium chloride or distilled water was necessary in order to facilitate its delivery by the pump. Under these conditions, 10 pore volumnes of the diluted sludge corresponded to the application of 2.5 cm (1 inch) of anaerobically digested sludge containing 2% solids (w/v). Table 4-8 describes the movement of poliovirus suspended in sludge diluted in 0.01 N calcium chloride. No virus breakthrough was detected prior to the seventh pore volume. At the tenth pore volume, only 0.01% of the total virus applied appeared in the soil effluent. The subsequent addition of two pore volumes of sterile rain water (which is the equivalent of 25 cm of rain) did not elute adsorbed viruses (see Table 4-8). In fact, at the 12th pore volume, the percent of total viruses applied which appeared in the leachate remained at 0.01. Table 4-9 displays the results

TABLE 4-7. Retention of poliovirus type 1 by a packed column of Eustis fine sand subsoil when suspended in 0.01 N $CaCl_2$

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration
1	0	0
2	0	0
3	4.2	0.06
4	0	0
5	4.2	0.06
6	0	0
7	0	0
8	25	0.3
9	21	0.3
10	0	0
11	0	0

[a]One pore volume for the column used equals 71 ml. The laboratory-packed column was 10 cm in length and 4.8 cm internal diameter. The sample of Eustis fine sand subsoil used consisted mainly of the A21 and A22 horizons (see Table 4-3). The column was conditioned with 5 pore volumes of 0.01 N $CaCl_2$. The solution was applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.).

[b]Poliovirus was seeded in the influent (i.e., 0.01 N $CaCl_2$) at at concentration of 7.3×10^3 PFU/ml. The conductivity of 0.01 N $CaCl_2$ was 1210 μmho/cm at 25°C and the pH was 6.4.

TABLE 4-8. Retention of poliovirus type 1 by a packed column of Red Bay sandy loam subsoil when suspended in anaerobically digested sludge diluted (1:50) with 0.01 N $CaCl_2$ and after subsequent application of rain water

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	% of Total PFU having been applied at each pore volume (cumulative)	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
	Sludge[c] diluted (1:50) with 0.01 N $CaCl_2$ and seeded with poliovirus				
1/3	0	0	0	1260	4.8
2/3	0	0	0	1280	5.2
1	0	0	0	1260	4.9
1 1/3	0	0	0	1270	4.9
1 2/3	0	0	0	1300	4.9
2	0	0	0	1320	5.0
2 1/3	0	0	0	1330	5.0
2 2/3	0	0	0	1340	4.9
3	0	0	0	1350	4.9
3 1/3	0	0	0	1350	4.9
3 2/3	0	0	0	1360	4.7
4	0	0	0	1360	4.9
4 1/3	0	0	0	1360	4.9
4 2/3	0	0	0	1360	4.9
5	0	0	0	1360	4.9
5 1/3	0	0	0	1360	4.9
5 2/3	0	0	0	1360	4.9
6	0	0	0	1360	4.9
6 1/3	0	0	0	1370	5.0
6 2/3	0	0	0	1370	5.0
7	7.5	0.1	0.005	1370	5.0
7 1/3	0	0	0.005	1370	5.0
7 2/3	0	0	0.005	1370	5.0
8	0	0	0.004	1370	5.0
8 1/3	15	0.2	0.01	1370	5.0

8 2/3	0	0	0.01	1370	5.0
9	0	0	0.01	1370	5.0
9 1/3	0	0	0.01	1370	5.0
9 2/3	0	0	0.01	1370	5.0
10	0	0	0.01	1370	5.0
		Shift to nonseeded rain water[d]			
10 1/3	0	0	0.01	1360	5.1
10 2/3	0	0	0.01	1360	5.1
11	0	0	0.01	1360	5.0
11 1/3	0	0	0.01	1200	5.1
11 2/3	0	0	0.01	800	5.0
12	7.5	0.1	0.01	400	5.1

[a]One pore volume for the column used equals 225 ml. The laboratory packed column was 29 cm in length and 4.8 cm internal diameter; the column was filled only 27 cm with soil (2 cm left on top for packed sludge). The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4 - 3). The column was conditioned with 5 pore volumes of 0.01 N $CaCl_2$. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.).

[b]Poliovirus was seeded in the influent (i.e., sludge diluted with 0.01 N $CaCl_2$) at a concentration of 7.3×10^3 PFU/ml.

[c]The anaerobically digested sludge (GDAN--see Table 3 - 2) used had a solids content of 2.0%, a conductivity of 3250 μmho/cm at 25°C and a pH of 8.3. The conductivity of sludge diluted (1:50) with 0.01 N $CaCl_2$ was 1350 μmho/cm at 25°C and the pH was 6.5.

[d]Rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4 - 2 for chemical characteristics of the rain water.

TABLE 4-9. Retention of poliovirus type 1 by a packed column of Red Bay sandy loam subsoil when suspended in anaerobically digested sludge diluted (1:50) with distilled water

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	% of Total PFU having been applied at each pore volume (cumulative)	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
0.5	0	0	0	66	6.0
1.0	0	0	0	72	5.6
1.5	0	0	0	87	5.9
2.0	0	0	0	94	5.8
2.5	0	0	0	95	6.1
3.0	0	0	0	97	5.7
3.5	0	0	0	96	5.6
4.0	0	0	0	93	6.1
4.5	0	0	0	92	5.8
5.0	0	0	0	92	5.8
5.5	0	0	0	90	5.8
6.0	0	0	0	89	5.8
6.5	0	0	0	87	5.8
7.0	0	0	0	88	5.9
7.5	0	0	0	86	5.9
8.0	0	0	0	88	5.8
8.5	0	0	0	93	5.9

[a]One pore volume for the column used equals 225 ml. The laboratory-packed column was 29 cm in length and 4.8 cm internal diameter; the column was filled only 27 cm with soil (2 cm left on top for packed sludge). The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The column was conditioned with 5 pore volumes of distilled water. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.).

[b]Poliovirus was seeded in the influent (i.e., sludge diluted with distilled water) at a concentration of 1.1×10^4 PFU/ml. The anaerobically digested sludge (GDAN--see Table 3-2) used had a solids content of 2.0%, a conductivity of 3250 μmho/cm at 25°C and a pH of 8.3. The conductivity of sludge diluted (1:50) with distilled water was 210 μmho/cm at 25°C and the pH was 7.4.

pertaining to the movement of poliovirus through the Red Bay sandy loam treated with sludge which had been diluted in distilled water. No virus breakthrough was detected in the soil leachates following percolation of 8.5 pore volumes. The application of sludge diluted with distilled water resulted in a gradual soil clogging and the percolation experiment was ended when only 8.5 pore volumes had been collected. In this particular experiment, the specific conductance was around 90 μmhos/cm and was probably sufficient to promote virus adsorption to the soil.

Eustis fine sand. The transport pattern of poliovirus suspended in anaerobically digested sludge diluted in distilled water or 0.01 N calcium chloride was next evaluated using columns of Eustis fine sand. As shown in Table 4-10, poliovirus suspended in sludge diluted in distilled water was found to rapidly move thorugh the soil and appear in the column effluent. Breakthrough occurred by the first pore volume (0.1%) and reached a maximum at the seventh pore volume of 39.9% of the influent poliovirus concentration. Fractions beyond the seventh pore volume could not be collected because of clogging of the column. The results for sludge diluted with 0.01 N calcium chloride, on the other hand, show no virus breakthrough by the ninth pore volume beyond which the column became clogged (see Table 4-10). Thus, it was observed that the change in the ionic composition of sludge when diluted in distilled water allowed for rapid virus transport through the soil column. It is postulated that a reduction in the specific conductance of the sludge diluted in distilled water resulted in poor virus

TABLE 4-10. Movement or retention of poliovirus type 1 when suspended in anaerobically digested sludge diluted (1:50) with distilled water or 0.01 N $CaCl_2$, respectively, and applied to 10 cm packed columns of Eustis fine sand subsoil

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
Column 1--applied sludge[c] diluted (1:50) with distilled water				
1	5.0×10^1	0.1	46	7.0
2	3.3×10^1	0.1	102	6.8
3	1.4×10^3	2.4	250	6.6
4	7.4×10^3	12.5	280	5.9
5	1.7×10^4	28.2	300	5.8
6	2.1×10^4	36.2	310	5.8
7	2.4×10^4	39.9	310	5.5
Column 2--applied sludge[c] diluted (1:50) with 0.01 N $CaCl_2$				
1	0	0	1230	5.1
2	0	0	1290	5.2
3	0	0	1310	5.2
4	0	0	1320	5.2
5	0	0	1360	5.3
6	0	0	1300	5.3
7	0	0	1250	5.4

TABLE 4-10. Continued.

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
8	0	0	1300	5.5
9	0	0	1400	5.4

[a]One pore volume for the columns used equals 71 ml. The laboratory-packed columns were 10 cm in length and 4.8 cm internal diameter. The sample of Eustis fine sand subsoil used consisted mainly of the A21 and A22 horizons (see Table 4-3). Columns 1 and 2 were conditioned with 5 pore volumes of distilled water and 0.01 N $CaCl_2$, respectively. All solutions were applied continuously to the columns at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.).

[b]Poliovirus was seeded in the influents of column 1 (i.e., sludge diluted with distilled water) and column 2 (i.e., sludge diluted with 0.01 N $CaCl_2$) at concentrations of 5.9×10^4 PFU/ml and 2.6×10^4 PFU/ml, respectively.

[c]The anaerobically digested sludge (PDAN--see Table 3-2) used had a solids content of 1.4% and a pH of 7.2. Chemical parameters were not measured for the sludge diluted (1:50) with distilled water or 0.01 N $CaCl_2$.

adsorption to the sludge and soil particles. Poliovirus retained by a Eustis fine sand column treated with sludge diluted with 0.01 N calcium chloride was not eluted with rain water (see Table 4-11). It appears, therefore, that adsorbed viruses in sludge-treated soils are not readily displaced and transported further down the soil profile by a solution low in ionic strength such as rain water.

The distribution of poliovirus within Eustis fine sand columns was investigated next. For this purpose, 29-cm columns were packed with 27 cm of this soil, and poliovirus was subsequently applied to the soil surface while suspended in sludge diluted (1:50, v/v) in distilled water or 0.01 N $CaCl_2$. Table 4-12 shows the transport patterns of poliovirus in 27-cm soil columns that had been treated with the diluted sludges. Similar trends were previously observed with the 10-cm columns (see Table 4-10). No virus breakthrough was observed in the column treated with sludge diluted with 0.01 N $CaCl_2$, whereas the column receiving sludge diluted with distilled water was found to display virus in the leachate after the third pore volume (see Table 4-12). The experiment was stopped by the fourth pore volume because the column receiving the sludge diluted with distilled water became clogged. The columns were subsequently sectioned (i.e., after allowing ponded water to soak in overnight) to study virus distribution within the soil (see Tables 4-13 and 4-14). It can be seen that in the column treated with sludge diluted with 0.01 N calcium chloride, poliovirus was found in the packed sludge and in the top 5 cm of soil (see (Table 4-13). The results for the column receiving sludge diluted with

TABLE 4-11. Retention of poliovirus type 1 by a 10 cm packed column of Eustis fine sand subsoil when suspended in anaerobically digested sludge diluted (1:50) with 0.01 N $CaCl_2$, and after subsequent application of rain water and 0.01 N $CaCl_2$

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
Sludge[c] diluted (1:50) with 0.01 N $CaCl_2$ and seeded with poliovirus				
1	0	0	1280	4.7
2	0	0	1335	4.9
3	0	0	1380	4.9
4	0	0	1400	5.0
5	0	0	1430	5.1
6	0	0	1430	5.2
7	0	0	1430	5.3
8	0	0	1420	5.2
9	0	0	1420	5.2
Shift to nonseeded rain water[d]				
10	0	0	1360	5.1
11	0	0	315	5.0
12	0	0	150	5.0
13	0	0	88	6.6
14	0	0	63	6.6

TABLE 4-11. Continued.

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
Shift to nonseeded 0.01 N $CaCl_2$[e]				
15	0	0	60	6.5
16	0	0	1010	5.2
17	22	0.1	1240	5.2
18	0	0	1320	5.2
19	0	0	1320	5.1
Shift to nonseeded rain water[d]				
20	0	0	1200	5.1
21	0	0	350	5.9
22	0	0	88	6.5
23	0	0	64	6.8
24	0	0	58	6.8

[a]One pore volume for the column used equals 71 ml. The laboratory-packed column was 10 cm in length and 4.8 cm internal diameter. The sample of Eustis fine sand subsoil used consisted mainly of the A21 and A22 horizons (see Table 4-3). The column was conditioned with 5 pore volumes of 0.01 N $CaCl_2$. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.).

[b]Poliovirus was seeded in the influent (i.e., sludge diluted with 0.01 N $CaCl_2$) at a concentration of 2.6×10^4 PFU/ml.

[c]The anaerobically digested sludge (PDAN--see Table 3-2) used had a solids content of 1.4% and a pH of 7.2. Chemical parameters were not measured for the sludge diluted (1:50) with 0.01 N $CaCl_2$.

[d]Rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.

[e]The conductivity and pH of 0.01 N $CaCl_2$ was 1210 μmho/cm at 25°C and 6.4, respectively.

TABLE 4-12. Movement or retention of poliovirus type 1 when suspended in anaerobically digested sludge diluted (1:50) with distilled water or 0.01 N $CaCl_2$, respectively, and applied to 27 cm packed columns of Eustis fine sand subsoil

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
Column 1--applied sludge[c] diluted (1:50) with distilled water				
0.5	0	0	29	6.6
1.0	0	0	29	6.3
1.5	0	0	51	6.2
2.0	0	0	61	6.2
2.5	0	0	104	6.5
3.0	0	0	77	6.5
3.5	2.5×10^2	1.8	78	5.6
4.0	2.7×10^2	1.9	--	--
Column 2--applied sludge[c] diluted (1:50) with 0.01 N $CaCl_2$				
0.5	0	0	1180	4.8
1.0	0	0	1270	4.8
1.5	0	0	1270	4.7
2.0	0	0	1300	4.8
2.5	0	0	1260	4.9
3.0	0	0	1300	4.9

TABLE 4-12. Continued.

No. of pore volumes[a] eluted	Poliovirus eluted (PFU/ml)	% of Influent[b] poliovirus concentration	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
3.5	0	0	1310	5.0
4.0	0	0	1310	5.0

[a]One pore volume for the columns used equals 192 ml. The laboratory-packed columns were 29 cm in length and 4.8 cm internal diameter; the columns were filled only 27 cm with soil (2 cm left on top for packed sludge). The sample of Eustis fine sand subsoil used consisted mainly of the A21 and A22 horizons (see Table 4-3). Columns 1 and 2 were conditioned with 5 pore volumes of distilled water and 0.01 N $CaCl_2$, respectively. All solutions were applied continuously to the columns at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). Following elution, the columns were sectioned as seen in Tables 4-13 and 4-14.

[b]Poliovirus was seeded in the influents of column 1 (i.e., sludge diluted with distilled water) and column 2 (i.e., sludge diluted with 0.01 N $CaCl_2$) at concentrations of 1.4×10^4 PFU/ml and 1.7×10^4 PFU/ml, respectively.

[c]The anaerobically digested sludge (PDAN--see Table 3-2) used had a solids content of 1.4% and a pH of 7.2. Chemical parameters were not measured for the sludge diluted (1:50) with distilled water or 0.01 N $CaCl_2$.

TABLE 4-13. Distribution of poliovirus type 1 in the soil profile of a 27-cm packed column of Eustis fine sand which had received virus-seeded, anaerobically digested sludge diluted (1:50) with 0.01 N $CaCl_2$

Depth in column[a] (cm)	Poliovirus recovered from soil[b]		
	PFU/g of wet soil	PFU/soil section	% of Total PFU applied[c]
Top Sludge[d]	38,200	2.1×10^5	1.6
0-3	1,000	9.9×10^4	0.8
3-5	80	8.1×10^4	0.6
5-7	0	0	0
7-9	0	0	0
9-11	0	0	0
11-13	0	0	0
13-15	0	0	0
15-17	0	0	0
17-19	0	0	0
19-21	0	0	0
21-23	0	0	0
23-25	0	0	0
25-27	0	0	0

[a]The laboratory-packed column was 29 cm in length and 4.8-cm internal diameter; the column was filled only 27 cm with soil (2 cm left on top for the packed sludge solids). The column was treated with virus-seeded diluted sludge as described in Table 4-12. The soil column was then sectioned and virus was eluted from each soil section.

TABLE 4-13. Continued.

[b]Each soil section was mixed well, and a 10-gram wet sample was taken and mixed with 20 ml of 3% beef extract, Tris buffered at pH 9.0. This mixture was then vortexed for 30 sec and sonicated for 3 min. The sample was then centrifuged at 1900 x g for 10 min at 4°C. The supernatant was subsequently assayed for viruses.

[c]The amount of virus applied to the soil column was 1.3×10^7 total PFU (see Table 4-12). Overall recovery of the virus applied was 3.0% [i.e., 3.0% found in the soil and 0% found in the soil leachates (see Table 4-12)].

[d]Refers to the sludge solids resting on top of the soil. The total amount of these solids was separated, subjected to the same virus elution method as the soil (added eluent at the proportion of 2 ml per gram of wet sludge solids) and was considered as one section.

TABLE 4-14. Distribution of poliovirus type 1 in the soil profile of a 27-cm packed column of Eustis fine sand which had received virus-seeded, anaerobically digested sludge diluted (1:50) with distilled water

Depth in column[a] (cm)	Poliovirus recovered from soil[b]		
	PFU/g of wet soil	PFU/soil section	% of Total PFU applied[c]
Top Sludge[d]	4,434	4.9×10^4	0.4
0-3	2,720	2.9×10^5	2.6
3-5	2,940	2.5×10^5	2.3
5-7	2,266	2.2×10^5	2.0
7-9	966	7.3×10^4	0.7
9-11	1,694	1.3×10^5	1.2
11-13	1,106	9.8×10^4	0.9
13-15	734	6.4×10^4	0.6
15-17	874	6.8×10^4	0.6
17-19	260	2.2×10^4	0.2
19-21	614	4.0×10^4	0.4
21-23	534	5.2×10^4	0.5
23-25	46	3.4×10^3	0.03
25-27	60	5.8×10^3	0.05

[a]The laboratory-packed column was 29 cm in length and 4.8-cm internal diameter; the column was filled only 27 cm with soil (2 cm left on top for the packed sludge solids). The column was treated with virus-seeded diluted sludge as described in Table 4-12. The soil column was then sectioned and virus was eluted from each soil section.

TABLE 4-14. Continued

[b]Each soil section was mixed well, and a 10-gram wet sample was taken and mixed with 20 ml of 3% beef extract, Tris buffered at pH 9.0. This mixture was then vortexed for 30 sec and sonicated for 3 min. The sample was then centrifuged at 1900 x g for 10 min at 4°C. The supernatant was subsequently assayed for viruses.

[c]The amount of virus applied to the soil column was 1.1×10^7 total PFU (see Table 4-12). Overall recovery of the virus applied was 13.0% [i.e., 12.5% found in the soil and 0.5% found in the soil leachates (see Table 4-12)].

[d]Refers to the sludge solids resting on top of the soil. The total amount of these solids was separated, subjected to the same virus elution method as the soil (added eluent at the proportion of 2 ml per gram of wet sludge solids) and was considered as one section.

distilled water, on the other hand, show that poliovirus was distributed throughout the length of the column with slightly higher concentrations in the top 3 cm of soil (2.6% of the total PFU applied--see Table 4-14). Thus, the results presented indicate that, because of the appropriate ionic environment, poliovirus was adsorbed in the top of the column receiving sludge diluted with 0.01 N calcium chloride. However, sludge diluted with distilled water affected the absorption process and allowed virus to move down the column, ultimately appearing in the effluent (see Table 4-12).

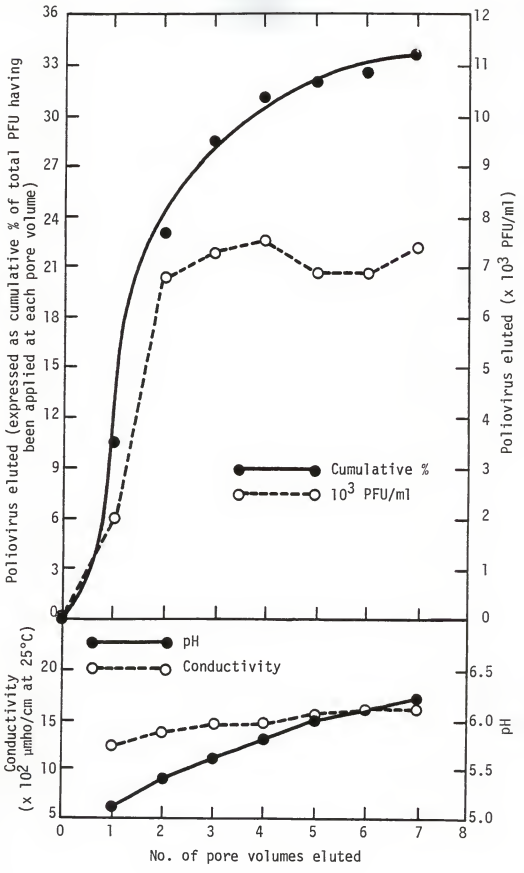
Poliovirus Suspended in Sludge Liquor

The centrifugation of anaerobically digested sludge resulted in a supernatant that will be referred to as sludge liquor. This liquor had a conductivity of 1580 μmhos/cm at 25°C and its pH was 8.1. Other characteristics of this liquor are displayed in Table 4-1. In order to stress the importance of sludge solids in virus movement through soils, soil column experiments were undertaken which involved viruses suspended in sludge liquor.

Red Bay sandy loam. Poliovirus was suspended in sludge liquor and subsequently was applied continuously to a 10-cm column of Red Bay sandy loam subsoil. In Figure 4-5, the breakthrough curve obtained is shown and it is seen that 33.7% of the total virus applied had appeared in the leachate by the seventh pore volume. The conductivity of the leachates did not vary significantly from the conductivity of the sludge liquor (i.e., 1580 μmho/cm at 25°C). The pH of the leachates varied from approximately 5.0 to 6.3. Moreover, it is seen in Table 4-1

FIGURE 4-5. Movement of poliovirus type 1 through a 10 cm packed column of Red Bay sandy loam subsoil when suspended in anaerobically digested sludge liquor

One pore volume for the column used equals 80 ml. The laboratory-packed column was 10 cm in length and 4.8 cm internal diameter. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The column was conditioned with 2 pore volumes of sludge liquor. Poliovirus was then suspended in the sludge liquor at a concentration of 1.9×10^4 PFU/ml and applied to the column. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The sludge liquor was produced by centrifuging anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3250 µmho/cm at 25°C and 8.3, respectively) at 14,000 x g for 10 min at 4°C. This procedure was performed again on the decanted supernatant and this yielded the clear sludge liquor. The conductivity of the sludge liquor was 1580 µmho/cm at 25°C and the pH was 8.1 (see Table 4-1 for other chemical parameters).


Poliovirus eluted (expressed as cumulative % of total PFU having been applied at each pore volume)
Poliovirus eluted (x 10³ PFU/ml)
Cumulative %
10³ PFU/ml
pH
Conductivity
Conductivity (x 10² µmho/cm at 25°C)
pH
No. of pore volumes eluted

that the sludge liquor contained high levels of Na, K, Ca, Mg, and soluble salts which would be conducive to virus adsorption to soil. In spite of this favorable ionic environment, a dramatic virus breakthrough (33.7%) occurred. These data support the contention that the sludge liquor contained substances which strongly interfered with virus adsorption to this soil. Furthermore, in this experiment, sludge solids which can bind viruses in the top of the soil matrix were not added. In a similar experiment, poliovirus was suspended in sludge diluted with sludge liquor brought to a final calcium chloride concentration of 0.01 N and subsequently applied to the soil column. In Figure 4-6, the breakthrough of poliovirus is seen to have been reduced to 22.6% (from 33.7% with only sludge liquor) as a direct result of the presence of sludge solids. When the length of the soil column was increased to 27 cm from 10 cm, there was a further decrease in poliovirus breakthrough from 22.6% to 8.1% (see Figure 4-7). Dilution of sludge in its own liquor prevents extreme changes in the sludge properties. For example, the pH was unchanged (8.3) and the conductivity was only slightly reduced from 3250 to 2600 μmho/cm at 25°C. A direct comparison can be made between the experiment in which 27-cm columns received sludge diluted with sludge liquor brought to a final $CaCl_2$ concentration of 0.01 N (Figure 4-7) and the experiment in which similar size columns were treated with sludge diluted in 0.01 N calcium chloride (Table 4-8). For these experiments, breakthroughs of poliovirus detected in the soil leachates at the tenth pore volume corresponded to 8.1% and 0.01% of the total virus applied, respectively. Quite clearly, the sludge liquor contained substances which interfered

FIGURE 4-6. Movement of poliovirus type 1 through a 10 cm packed column of Red Bay sandy loam subsoil when suspended in anaerobically digested sludge diluted (1:50) with sludge liquor containing 0.01 N $CaCl_2$

One pore volume for the column used equals 80 ml. The laboratory-packed column was 10 cm in length and 4.8 cm internal diameter. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The column was conditioned with 2 pore volumes of sludge liquor containing 0.01 N $CaCl_2$. Poliovirus was then suspended in the diluted sludge at a concentration of 4.0×10^3 PFU/ml and applied to the column. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The anaerobically digested sludge (GDAN--see Table 3-2) used had a solids content of 2.0%, a conductivity of 3250 μmho/cm at 25°C and a pH of 8.3. The conductivity of sludge diluted (1:50) with sludge liquor containing 0.01 N $CaCl_2$ was 2500 μmho/cm at 25°C and the pH was 7.5. The sludge liquor was produced by centrifuging GDAN sludge at 14,000 x g for 10 min at 4°C. This procedure was performed again on the decanted supernatant and this yielded the clear sludge liquor (see Table 4-2 for chemical parameters).

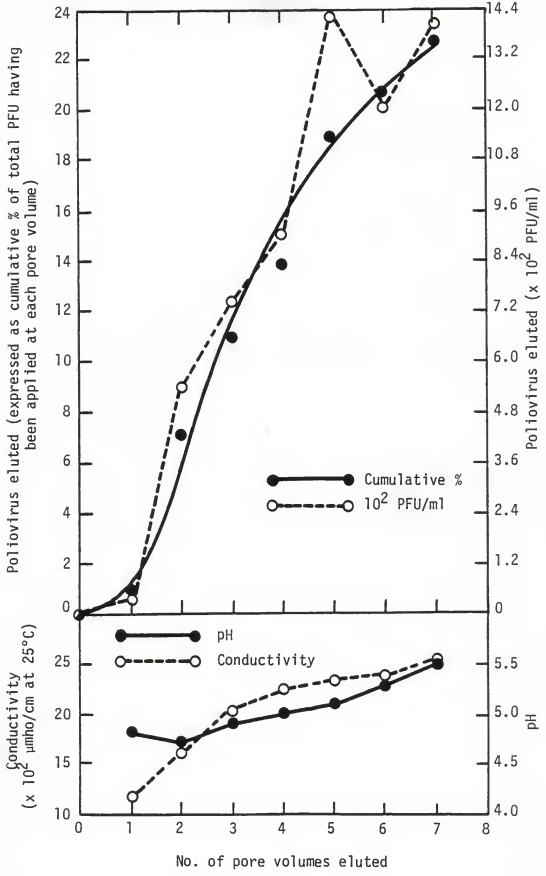

Poliovirus eluted (expressed as cumulative % of total PFU having been applied at each pore volume)
Poliovirus eluted (x 10² PFU/ml)
Cumulative %
10² PFU/ml
pH
Conductivity
Conductivity (x 10² µmho/cm at 25°C)
pH
No. of pore volumes eluted

FIGURE 4-7. Movement of poliovirus type 1 through a 27 cm packed column of Red Bay sandy loam subsoil when suspended in anaerobically digested sludge diluted (1:50) with sludge liquor containing 0.01 N $CaCl_2$

One pore volume for the column used equals 225 ml. The laboratory-packed column was 29 cm in length and 4.8 cm internal diameter; the column was filled only 27 cm with soil (2 cm left on top for packed sludge). The sample of Red Bay sandy loam subsoil used consisted mainly of A2 and B1t horizons (see Table 4-3). The column was conditioned with 2 pore volumes of sludge liquor containing 0.01 N $CaCl_2$. Poliovirus was then suspended in the diluted sludge at a concentration of 7.0×10^3 PFU/ml and applied to the column. All solutions were applied continuously to the column at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The anaerobically digested sludge (GDAN--see Table 3-2) used had a solids content of 2.0%, a conductivity of 3250 µmho/cm at 25°C and a pH of 8.3. The conductivity of sludge diluted (1:50) with sludge liquor containing 0.01 N $CaCl_2$ was 2600 µmho/cm at 25°C and the pH was 8.3. The sludge liquor was produced by centrifuging GDAN sludge at 14,000 x g for 10 min at 4°C. This procedure was performed again on the decanted supernatant and this yielded the clear sludge liquor (see Table 4-2 for chemical parameters).

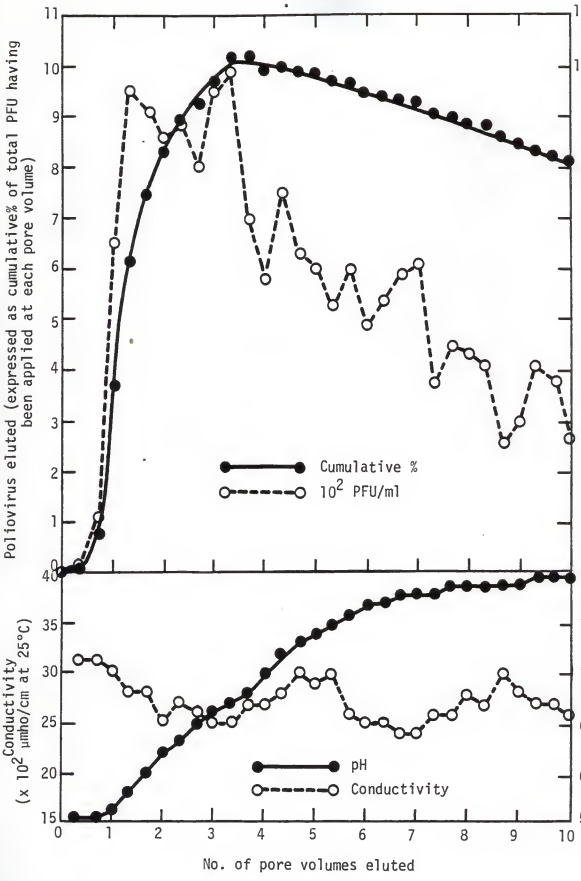
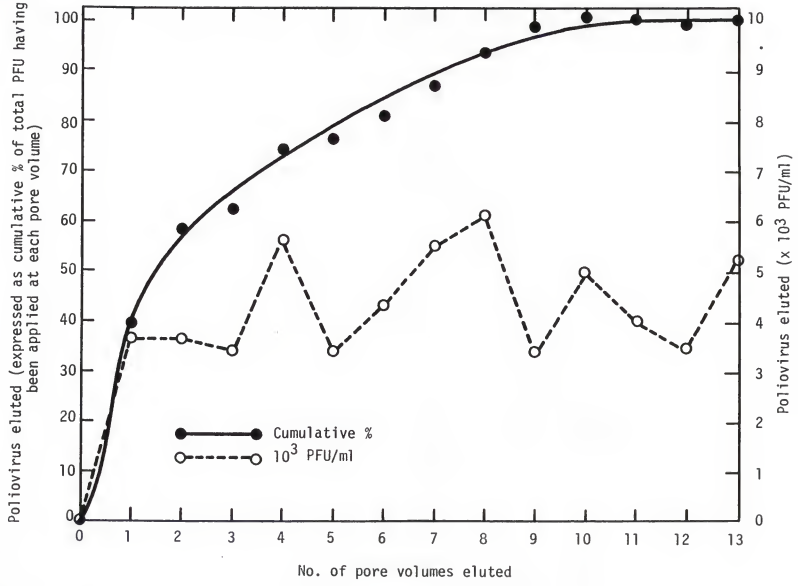

Poliovirus eluted (expressed as cumulative% of total PFU having been applied at each pore volume)
Poliovirus eluted (x 10² PFU/ml)
Cumulative %
10² PFU/ml
Conductivity (x 10² µmho/cm at 25°C)
pH
pH
Conductivity
No. of pore volumes eluted

FIGURE 4-8. Movement of poliovirus type 1 through a 10 cm packed column of Eustis fine sand subsoil when suspended in filtered-pH adjusted lagoon sludge liquor

One pore volume for the column used equals 71 ml. The laboratory-packed column was 10 cm in length and 4.8 cm internal diameter. The sample of Eustis fine sand subsoil used consisted mainly of the A21 and A22 horizons (see Table 4-3). The column was conditioned with 2 pore volumes of filtered-pH adjusted sludge liquor. The poliovirus was then suspended in the filtered-pH adjusted sludge liquor at a concentration of 4.5×10^3 PFU/ml and applied to the column. All solutions were applied continuously to the column at approximately 3 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The sludge liquor was produced by centrifuging lagoon sludge (LAG--see Table 3-2; solids content, conductivity and pH equal to 2.9%, 3500 μmho/cm at 25°C and 6.9, respectively) at 14,000 x g for 10 min at 4°C. The sludge liquor (i.e., the supernatant from the previous centrifugation) was passed through a series of 0.45 and 0.25 μm Filterite filters (Filterite Corp., Timonium, Md.) in a 47 mm holder and then adjusted to pH 8.0 using 0.01 N NaOH. The conductivity of the filtered-pH adjusted sludge liquor was 1800 μmho/cm at 25°C.


Poliovirus eluted (expressed as cumulative % of total PFU having been applied at each pore volume)
Poliovirus eluted (x 10^3 PFU/ml)
No. of pore volumes eluted
Cumulative %
10^3 PFU/ml
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8
9
11
12
13

with the adsorption of poliovirus to sludge and soil particles. Furthermore, Figure 4-7 shows that the passage of ten pore volumes (2250 ml) of sludge diluted with sludge liquor brought to a final $CaCl_2$ concentration of 0.01 N resulted in the gradual increase in the soil solution pH from 5.8 to the pH of the leaching solution, 8.3. Thus, the soil was unable to buffer the pH and this increase in pH into the basic range further prevented viral adsorption to the soil.

Eustis fine sand. Experiments involving poliovirus suspended in sludge liquor were also performed using Eustis fine sand columns. The sludge liquor employed in these studies was produced by centrifuging lagoon sludge (LAG--see Table 3-2; 2/3 anaerobically digested and 1/3 aerobically digested sludge), and subsequently passing the resulting supernatant through a series of 0.45 and 0.25 μm Filterite filters and then adjusting the sludge liquor to pH 8.0. The filtration procedure did not remove all bacterial cells from the sludge liquor. This was confirmed by microscopic examination of the sludge liquor at a magnification of 1000x. Figure 4-8 shows that poliovirus breakthrough occurred at the first pore volume, and by the tenth pore volume, 100% of the applied virus had appeared in the leachate from a 10-cm column of this soil. Further research by Overman et al. (unpublished data) has confirmed that this sludge liquor strongly interferes with the adsorption of poliovirus type 1 and echovirus type 4 to the Eustis fine sand. Moreover, these investigators demonstrated that this sludge liquor was not able to elute substantial numbers of previously adsorbed viruses (i.e., poliovirus and echovirus).

The mechanisms(s) of sludge liquor interference with virus adsorption to soils is (are) not well understood. It is known that water-soluble "humic substances" interfere with the sorptive capacity of soil and sediments toward viruses. The decrease in virus retention is due to humic fractions with a molecular weight of less than 50,000 (Bitton et al. 1977; Scheuerman et al. 1979). Anaerobically digested sludge contains fulvic acid fractions (Baham et al. 1978; Holtzclaw et al. 1976, 1978; Sposito and Holtzclaw 1977; Sposito et al. 1976, 1978) that are known to complex Ca^{+2} ions, an important and ubiquitous metal cation in soil solution (Sposito et al. 1978). This complexation phenomenon may aid in the inhibition of virus adsorption to soils. Other mechanisms are probably involved in this inhibition process. When poliovirus was suspended in sludge liquor that had been filtered through a 0.22 μm Millipore filter, the percent breakthrough of this virus in Eustis fine sand columns was significantly reduced (i.e., 4%) as compared to the breakthrough (34%) following the application of Filterite-treated sludge liquor (Overman et al., unpublished data). Microscopic examination showed that Filterite-treated sludge liquor contained substantial numbers of bacterial cells whereas no bacteria were found in Millipore-treated sludge liquor. It is then possible that these bacterial cells may compete with viruses for adsorption sites on the surface of the soil particles. This is a mere speculation that needs to be demonstrated under more controlled conditions. Competitive adsorption between viruses and bacteria has not been reported in the literature.

The sludge liquor employed in these experiments represents an artificial system probably never encountered in the environment. However, under field conditions, rain water may leach the sludge liquor from the applied sludge, which then may affect virus transport through the soil. The implications of this possible phenomenon in field situations deserve further investigation.

Poliovirus Suspended in Undiluted Anaerobically Digested Sludge

The movement of sludge-associated viruses through soil was next studied under more realistic conditions. Poliovirus was added to undiluted anaerobically digested sludge while stirring the mixture on a magnetic stirrer. One inch (2.5 cm) of seeded sludge was then applied on top of undisturbed cores of Eustis fine sand and Red Bay sandy loam. The sludge was allowed to soak in (in one experiment the sludge was allowed to air dry for 24 hours) and then worked under. The soil cores were then eluted with either 0.01 N $CaCl_2$ or rain water.

Red Bay sandy loam. Undisturbed soil cores of Red Bay sandy loam were treated with poliovirus-seeded sludge as described above. The applied sludge was allowed to air dry for 24 hours, under field conditions, before being mixed with the top 2.5 cm of soil to simulate field practices. The soil columns were then eluted with three to four pore volumes of rain water. Table 4-15 shows that there was a virus breakthrough in the second or the first pore volume collected. This virus breakthrough represented 0.1 and 0.2% of the total PFU applied to

TABLE 4-15. Retention of poliovirus type 1 by undisturbed cores of Red Bay sandy loam following the application of 2.5 cm of seeded anaerobically digested sludge (air dried 24 hrs) and the subsequent elution with rain water

No. of pore volumes[a] eluted	Poliovirus eluted (total PFU)	% of Total PFU applied[b] (cumulative)	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
Column 1				
1/3	0	0	245	4.4
2/3	0	0	195	4.7
1	0	0	125	4.8
1 1/3	0	0	106	5.1
1 2/3	1.1×10^3	0.2	80	5.2
2	0	0.2	82	5.1
2 1/3	0	0.2	77	5.1
2 2/3	0	0.2	72	5.0
3	0	0.2	66	5.0
3 1/3	0	0.2	66	5.0
Column 2				
1/3	5.2×10^2	0.1	232	4.8
2/3	0	0.1	177	5.2
1	0	0.1	157	5.5
1 1/3	0	0.1	115	6.0
1 2/3	0	0.1	98	5.6
2	0	0.1	87	5.4
2 1/3	0	0.1	75	5.6
2 2/3	0	0.1	69	5.5
3	0	0.1	62	5.4
3 1/3	0	0.1	58	5.5

TABLE 4-15. Continued.

No. of pore volumes[a] eluted	Poliovirus eluted (total PFU)	% of Total[b] PFU applied (cumulative)	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
3 2/3	0	0.1	54	5.6
4	0	0.1	54	5.7
4 1/3	0	0.1	49	6.4

[a]One pore volume for these cores equals 471 ml. The undisturbed soil cores were 54 cm in length and 5.0 cm internal diameter; consists of the A1, A2, B1t and B21t horizons of the Red Bay sandy loam (see Table 4-3). The cores were not conditioned.

[b]One inch or 2.5 cm (51.6 ml) of anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3250 μmho/cm at 25°C and 8.3, respectively) seeded with a total of 5.1 x 10^5 PFU of poliovirus was applied to each of the cores. The cores were then placed on the roof of the Environmental Engineering Sciences building at the University of Florida, Gainesville. The applied sludge was allowed to air dry for 24 hrs and then was worked under 2.5 cm. Elution with rain water was subsequently undertaken. This solution was applied from an inverted, self-regulated, 1 liter Erlenmeyer flask set to maintain a 2.5 cm hydraulic head on the cores. The flow rate through the cores was measured at 2.4 ml/min. The rain water was collected next to the Environmental Engineering Sciences building. See Table 4-2 for chemical characteristics of the rain water.

the soil columns. Afterwards, no virus was detected in the leachates, even after the passage of 77.5 to 93 cm of rain (equivalent to 1,570 to 1,884 ml of rain water). The observed breakthrough of poliovirus when the first pore volume had percolated through column 2 is not surprising due to the fact that the columns were not initially saturated. A soil column treated with 2.5 cm of sludge and subsequently eluted with 0.01 N calcium chloride was used as a control. From Table 4-16, it is clear that no virus could be detected in the column leachates. As discussed earlier, the presence of calcium chloride in the soil solution readily enhances virus adsorption to the soil matrix. However, rain water was able to transport 0.1 to 0.2% of the total applied viruses through the soil profile. This breakthrough would probably be lower if the soil was allowed to dry for a longer period of time under field conditions (see Chapter V).

The results presented above show that the Red Bay sandy loam studied is effective in retaining viruses during sludge application. Virus associated with sludge solids will be retained at the surface of the soil matrix and will be inactivated with time due to environmental factors (e.g., temperature, drying and solar radiation). In Table 3-6, the adsorption of poliovirus to anaerobic sludge solids during a 12-hour contact period is found to range from 52.8% to 69.1%. Thus, the effectiveness of virus retention by soils during sludge application is partly attributed to the capacity of sludge solids to bind viruses in the top of the soil profile. However, viable "free" virus (i.e., viruses not associated with sludge solids or dissociated

TABLE 4-16. Retention of poliovirus type 1 by an undisturbed core of Red Bay sandy loam following the application of 2.5 cm of seeded anaerobically digested sludge and the subsequent elution with 0.01 N $CaCl_2$

No. of pore volumes[a] eluted	Poliovirus eluted (total PFU)	% of Total PFU applied[b] (cumulative)	Conductivity of pore volume collected (μmho/cm at 25°C)	pH of pore volume collected
1/3	0	0	650	4.5
2/3	0	0	1360	4.5
1	0	0	1570	4.8
1 1/3	0	0	1660	5.2
1 2/3	0	0	1720	4.8
2	0	0	1750	5.0
2 1/3	0	0	1760	4.9
2 2/3	0	0	1770	4.9
3	0	0	1770	4.8
3 1/3	0	0	1770	4.8
3 2/3	0	0	1770	4.8
4	0	0	1770	4.8
4 1/3	0	0	1770	4.7

[a]One pore volume for this core equals 471 ml. The undisturbed soil core was 54 cm in length and 5.0 cm internal diameter; consists of the A1, A2, B1t and B21t horizons of the Red Bay sandy loam (see Table 4-3). The core was not conditioned.

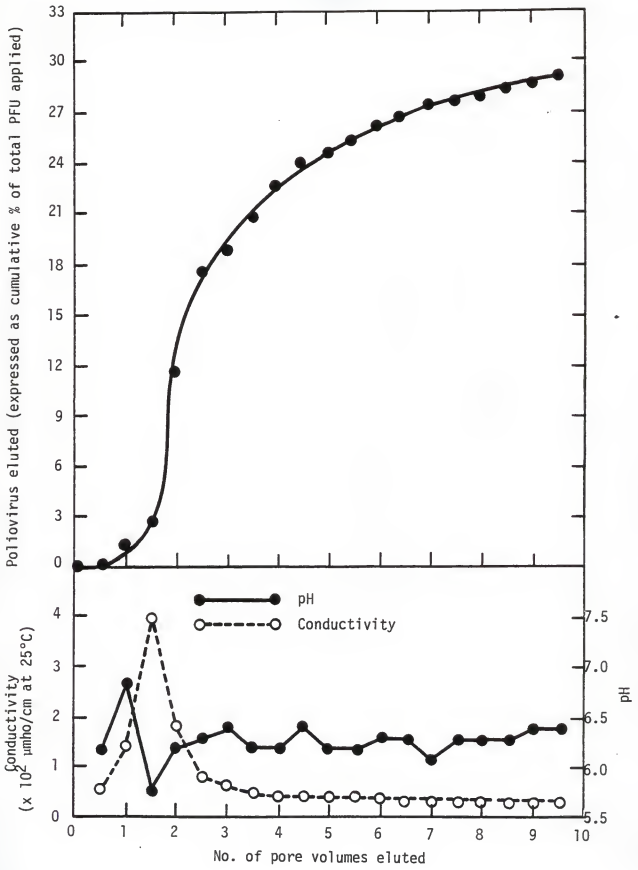
[b]One inch or 2.5 cm (51.6 ml) of anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3250 μmho/cm at 25°C and 8.3, respectively) seeded with a total of 2.2×10^5 PFU of poliovirus was applied to the core, allowed to soak in and then, worked under 2.5 cm. Elution with 0.01 N $CaCl_2$ (conductivity and pH equal to 1210 μmho/cm at 25°C and 6.4, respectively) was subsequently undertaken. This solution was applied from an inverted, self-regulated, 1 liter Erlenmeyer flask set to maintain a 2.5 cm hydraulic head on the core. The flow rate through the core was measured at 3.5 ml/min.

from sludge solids as a result of changes in the physico-chemical properties within the soil matrix--see Table 3-6) will move in the soil solution or be retained by the soil particles as govered by pH, flow rate, conductivity, and the presence of soluble organic materials (Bitton 1975; Gerba et al. 1975). Of particular importance in the retention of these "free" viruses by the soil is the nature of the soil itself and, more specifically, the clay content of the soil. Due to their large surface area, the clay minerals in soils comprise the fraction most active in retaining viruses (Carlson et al. 1968). The Red Bay sandy loam studied displayed a clay content ranging from 13.6% in the A1 horizon to 36.2% in the B21t horizon (see Table 4-3). The dominant clay in the A1, A2, and B1t horizons was vermiculite, while in the B21t horizon, it was gibbsite. The retention of "free" poliovirus during sludge application to the Red Bay sandy loam is attributed to the adsorptive capacity of the clay fraction found throughout the soil profile and accumulated in the deeper horizons (e.g., B1t and B21t). Moreover, this soil contained iron oxides which are also effective in retaining viruses (Bitton 1980a). The interaction between iron oxides and viruses in soils deserves further study.

Eustis fine sand. Similar application of sludge to an undisturbed core of Eustis fine sand and subsequent elution with rain water resulted in the breakthrough of 29.3% of the total (5.7×10^5 PFU) poliovirus applied (see Figure 4-9). A peak in conductivity (400 µmho/cm at 25°C) was found at the 1.5 pore volume and this was probably due to sludge leachates passing through the soil matrix. This

FIGURE 4-9. Movement of poliovirus type 1 through an undisturbed core of Eustis fine sand (conditioned with rain water) following the application of 2.5 cm of seeded anaerobically digested sludge and the subsequent elution with rain water

One pore volume for the core used equals 234 ml. The undisturbed soil core was 33 cm in length and 5.0 cm internal diameter; consists of the Ap and A21 horizons of the Eustis fine sand (see Table 4-3). The core was initially conditioned with 5 pore volumes of rain water. One inch or 2.5 cm (51.6 ml) of anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3250 μmho/cm at 25°C and 8.3, respectively) seeded with a total of 5.7×10^5 PFU of poliovirus was applied to the core, allowed to soak in and then, was worked under 2.5 cm. Elution with rain water was subsequently undertaken. This solution was applied from an inverted, self-regulated, 1 liter Erlenmeyer flask set to maintain a 2.5 cm hydraulic head on the core. The flow rate through the core was measured at 3.9 ml/min. The rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.


Poliovirus eluted (expressed as cumulative % of total PFU applied)
33
30
27
24
21
18
15
12
9
6
3
0
pH
Conductivity
Conductivity
(x 10² µmho/cm at 25°C)
4
3
2
1
0
7.5
7.0
6.5
6.0
5.5
pH
0
1
2
3
4
5
6
7
8
9
10
No. of pore volumes eluted

undisturbed core was initially conditioned with rain water. When an identical, undisturbed core was conditioned with 0.01 N $CaCl_2$, only 10.6% of the total poliovirus applied was detected in the effluent by the 8.5 pore volume (see Figure 4-10). Conditioning with calcium chloride enhanced the adsorption of viruses to the soil. Thus, it was found that the nature of the conditioning solution can affect the virus breakthrough pattern later obtained.

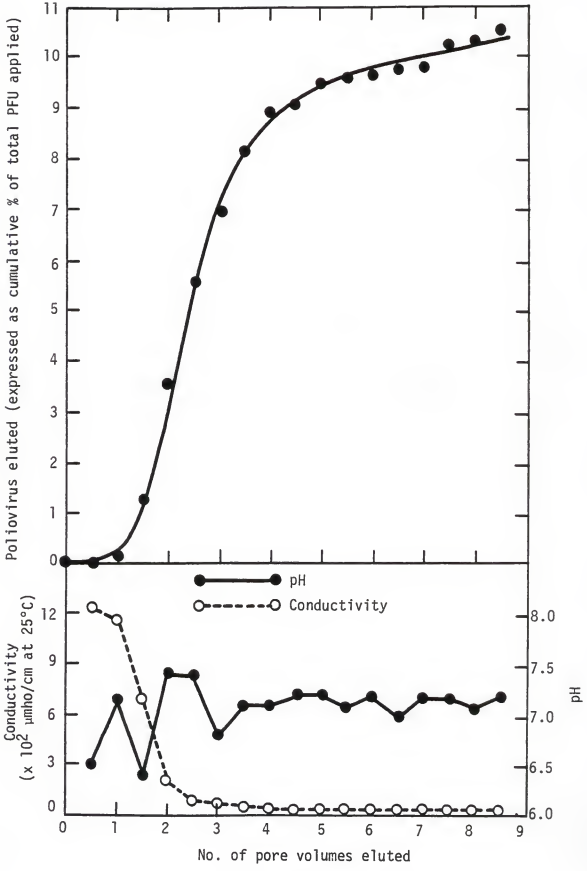
It appears then that Eustis fine sand, under saturated flow, does not retain viruses effectively (in Chapter V,data are presented on virus transport through Eustis fine sand cores under unsaturated flow and under field conditions). There is a dramatic difference between the virus "retention potential" of the Red Bay sandy loam (99.8%) and the Eustis fine sand (70.7%). It is postulated that the low adsorptive capacity of the Eustis fine sand is a direct result of its low clay content (only 3.2%, average for the Ap and A21 horizons, see Table 4-3). In the case of the Eustis fine sand, sludge application could lead to ground water contamination with pathogenic viruses. However, the results presented for the Red Bay sandy loam indicate that, under appropriate conditions, sludge application could be undertaken without threatening the quality of ground water with viruses.

Conditioned-Dewatered Sludge

At many wastewater treatment plants around the country, digested sludges are conditioned with polymers prior to dewatering. At the Main Street wastewater treatment plant in Gainesville, Florida,

FIGURE 4-10. Movement of poliovirus type 1 through an undisturbed core of Eustis fine sand (conditioned with 0.01 N $CaCl_2$) following the application of 2.5 cm of seeded anaerobically digested sludge and the subsequent elution with rain water

One pore volume for the core used equals 234 ml. The undisturbed soil core was 33 cm in length and 5.0 cm internal diameter; consists of the Ap and A21 horizons of the Eustis fine sand (see Table 4-3). The core was initially conditioned with 5 pore volumes of 0.01 N $CaCl_2$ (conductivity and pH equal to 1210 μmho/cm at 25°C and 6.4, respectively). One inch or 2.5 cm (51.6 ml) of anaerobically digested sludge (GDAN--see Table 3-2; solids content, conductivity and pH equal to 2.0%, 3250 μmho/cm at 25°C and 8.3, respectively) seeded with a total of 9.8×10^5 PFU of poliovirus was applied to the core, allowed to soak in and then, was worked under 2.5 cm. Elution with rain water was subsequently undertaken. This solution was applied from an inverted, self-regulated, 1 liter Erlenmeyer flask set to maintain a 2.5 cm hydraulic head on the core. The flow rate through the core was measured at 3.9 ml/min. The rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.


Poliovirus eluted (expressed as cumulative % of total PFU applied)
pH
Conductivity
Conductivity (x 10² µmho/cm at 25°C)
pH
No. of pore volumes eluted

for example, digested sludge is conditioned with a cationic polymer (Hercofloc #871, Hercules Co., Atlanta, Ga.) and subsequently dewatered by centrifugation. The fate of poliovirus seeded in anaerobically digested sludge was determined following conditioning and dewatering of sludge as described above. As shown in Table 4-17, in two experiments, 72.2% and 89.3% of poliovirus initially added to the sludge was found in the conditioned-dewatered sludge. Moreover, in both experiments, 99.9% of the virus recovered from the conditioned-dewatered sludge was found associated with the sludge solids (see Table 4-18). The application of the conditioned-dewatered sludge containing poliovirus to columns of Red Bay sandy loam and the subsequent elution with rainwater did not result in any virus breakthrough (see Table 4-19). These results show that viruses present in conditioned-dewatered sludge are effectively retained by soils.

Chemical Sludges

In addition to biological sludges, chemical sludges may also be produced during wastewater treatment. These sludges may be produced during primary treatment when coagulation (using alum, ferric chloride, or lime) is combined with sedimentation to upgrade the removal efficiency of the treatment process (i.e., intermediate treatment) or during advanced wastewater treatment (tertiary treatment) (Malina et al. 1976). Previous research has demonstrated that viruses are concentrated in alum and ferric chloride sludges (Lund and Rønne 1973; Wolff et al. 1974). However, in lime sludges, viruses have been found to be effectively inactivated (Lund and Rønne 1973; Sattar et al. 1976).

TABLE 4-17. Association of poliovirus type 1 with anaerobically digested sludge conditioned with a cationic polymer and subsequently dewatered by centrifugation

Experiment no.	Virus[a] in 1000 ml[b] of sludge (total PFU)	Virus in sludge supernatant		Virus in unfractionated[c], dewatered sludge			
		Total PFU	Recovery[d] (%)	Total PFU	Recovery[d] (%)	Sludge volume (ml)	Sludge solids[e] content (%)
1	2.8×10^7	2.4×10^6	8.6	2.5×10^7	89.3	320	5.1
2	3.6×10^7	2.4×10^6	6.7	2.6×10^7	72.2	310	4.7

[a]Virus in the sludge before the addition of polymer. The cationic polymer used was Hercofloc #871 (Hercules Co., Atlanta, Georgia) and it was used at a concentration of 1200 mg/l in the sludge.

[b]The anaerobically digested sludge (GDAN--see Table 3-2) used had a solids content of 2.0%, a conductivity of 3950 μmho/cm at 25°C and a pH of 6.0. The sludge was autoclaved prior to use. The sludge was conditioned-dewatered as follows. The polymer was added to 1000 ml of virus-seeded sludge while mixing rapidly on a magnetic stirrer. Mixing was continued slowly for an additional 5 min. The entire sample was then centrifuged at 320 x g for 10 min at 25°C. The supernatant was decanted, assayed for viruses and discarded. The dewatered sludge produced was then assayed for viruses. The dewatered sludges from experiments no. 1 and 2 were applied to Red Bay sandy loam columns 1 and 2, respectively, as shown in Table

[c]The sludge solids were not separated prior to assaying.

[d]Percent recoveries were calculated based on the amount of viruses (total PFU) present in the sludge before the addition of polymer at 100%.

[e]Sludge solids content was expressed as a percentage on a weight to volume basis.

TABLE 4-18. Association between poliovirus type 1 and conditioned-dewatered sludge solids

Experiment no.	Virus in unfractionated[a] sludge[b] (total PFU)	Virus in sludge supernatant[c] (total PFU)	Viable unadsorbed[d] virus (%)	Solids-associated[e] virus (%)
1	2.5×10^7	1.1×10^4	0.04	99.9
2	2.6×10^7	3.1×10^4	0.1	99.9

[a]The sludge solids were not separated prior to assaying.

[b]The methods used to produce the dewatered sludges and to determine the amount of viruses present in the dewatered sludges are described in Table 4-17.

[c]The sludge was clarified by centrifugation at 1400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[d]The "viable unadsorbed virus (%)" values were calculated as shown in the Materials and Methods section.

[e]The "solids-associated virus (%)" values were estimated as shown in the Materials and Methods section.

TABLE 4-19. Retention of poliovirus type 1 by packed columns of Red Bay sandy loam subsoil following the application of conditioned-dewatered sludge and the subsequent leaching with rain water

Total no. of pore volumes[a] eluted	Sludge[b] volume applied (ml)	Sludge solids applied (g)	Total no. of poliovirus, PFU		Range of conductivity values for pore volumes collected (μmho/cm at 25°C)	Range of pH values for pore volumes collected
			Contained in the sludge applied	Passing through the soil[c]		
Column 1						
10.0	17	0.9	1.3×10^6	0	41 - 148	5.5 - 6.7
Column 2						
10.0	17	0.8	1.4×10^6	0	37 - 175	5.6 - 6.5

[a]One pore volume for these columns equals 225 ml. The laboratory packed columns were 29 cm in length and 4.8 cm internal diameter; the columns were filled only 27 cm with soil (2 cm left on top for packed sludge), and were conditioned with 2 pore volumes of rain water. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3).

[b]The methods used to produce the dewatered sludges and to determine the amount of viruses present in the dewatered sludges are described in Table 4-17. The dewatered sludges were applied to the soil columns, allowed to soak in, and then, worked under 2.5 cm. Elution with rain water was subsequently undertaken. The rain water was applied continuously to the columns at approximately 5 ml/mn using a peristaltic pump (Buchler, Fort Lee, N.J.). The rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.

[c]Soil leachates were collected in 1/2 pore volume fractions and assayed individually for viral infectivity. Concentration of soil leachates was not performed.

Experiments were undertaken to assess the capacity of the Red Bay sandy loam subsoil to retain viruses following the application of chemical sludges (alum, ferric chloride, and lime). The aim of this research was to determine if there is a risk of groundwater contamination with viruses when chemical sludges are disposed on land.

The chemical sludges (alum, ferric chloride, and lime) were precipitated from poliovirus-seeded, raw sewage. As shown in Table 4-20, variable fractions of the seeded viruses were recovered from the chemical sludges produced (22.6% to 95.6% for alum sludges, 9.8% to 13.3% for ferric chloride sludges, and 0% to 0.1% for lime sludges). Since the viruses became embedded in the sludges produced during the flocculation process, it is likely that not all viruses present in the sludges were recovered. It is generally believed that embedded viruses are difficult to elute. It does appear, however, that due to the high pH of the lime sludges, most of the viruses originally seeded in the raw sewage were inactivated (see Tables 4-20 and 4-21). The association between poliovirus and chemical sludge solids was then evaluated. It was found that from 97% to 100% of input virus was associated with alum, ferric chloride, and lime sludge solids (Table 4-21). In one sample (experiment no. 2) of lime sludge, all viruses were inactivated due to the high pH (pH 11.3) generated during the process. The application of these virus-seeded sludges to soil columns of Red Bay sandy loam did not result in any virus breakthrough following leaching with two to ten pore volumes of rainwater (Table 4-22). It is worth stressing that no virus could

TABLE 4-20. Association of poliovirus type 1 with chemical sludges precipitated from virus-seeded, raw sewage

Sludge[a] type	Experiment no.	Virus in sewage[b]			Virus in supernatant		Virus in unfractionated[d] sludge				Sludge was	
		Before addition of coagulant	After addition of coagulant[c]									
		Total PFU	Total PFU	Recovery[e] (%)	Total PFU	Recovery[e] (%)	Total PFU	Recovery[e] (%)	Sludge volume (ml)	Sludge solids[f] content (%)	Limed	Applied to soil column no.
Alum	1	5.3×10^7	--[g]	--	9.4×10^6	17.7	1.2×10^7	22.6	20	--	No	1, Table 4-22
	2	4.5×10^7	4.5×10^7	100.0	1.6×10^6	3.6	4.3×10^7	95.6	50	0.3	No	2, Table 4-22
	3	3.9×10^7	--	--	4.7×10^5	1.2	1.1×10^7	28.2	21	1.3	Yes, Table 4-23	--
Ferric chloride	1	4.3×10^7	7.7×10^6	17.9	4.0×10^5	0.9	4.7×10^6	10.9	31	0.5	No	1, Table 4-22
	2	4.5×10^7	7.3×10^6	16.2	9.3×10^5	2.1	4.4×10^6	9.8	33	0.4	No	2, Table 4-22
	3	3.9×10^7	9.8×10^6	25.1	9.3×10^5	2.4	5.2×10^6	13.3	31	0.7	Yes, Table 4-23	--
Lime	1	5.8×10^7	--	--	1.0×10^5	0.2	7.5×10^4	0.1	30	--	No	1, Table 4-22
	2	4.5×10^7	0	0	0	0	0	0	37	1.0	No	2, Table 4-22

[a]The chemical sludges were precipitated from 1000 ml of virus-seeded, raw sewage using the concentrations of coagulants shown below. Virus assays were made before and after the addition of coagulants. Following the addition of alum and ferric chloride, the pHs of the solutions were adjusted to 6.0 and 5.0, respectively, in order to achieve maximum flocculation. The coagulant, lime, was added until pHs of 11.1 and 11.3 were achieved in experiments no. 1 and no. 2, respectively. Following the addition of the coagulants, the sewage samples were mixed on a magnetic stirrer rapidly for 10 min and slowly for 5 min. The flocculated sewage samples were then transferred to Imhoff cones and 60 min was allowed for the formation and settling of the sludges. The supernatants in the Imhoff cones were assayed for viruses and discarded. The sludges produced were then assayed for viruses.

[b]The sewage used was sampled at the University of Florida campus sewage treatment plant (only used for experiments no. 1-alum and no.1-lime; conductivity and pH equal to 520 μmho/cm at 25°C and 9.0, respectively), or at the Main street sewage treatment plant of Gainesville, Florida (used for all other experiments; conductivity and pH equal to 770 μmho/cm at 25°C and 8.9, respectively). The sewage samples were sterilized by autoclaving prior to use.

[c]The final concentrations in sewage of the coagulants used were 300 mg/l of $Al_2(SO_4)_3 \cdot 18\ H_2O$, 50 mg/l of $FeCl_3$, and 150 or 250 mg/l of $Ca(OH)_2$ (for experiments no. 1 and no. 2, respectively).

[d]The sludge solids were not separated prior to assaying.

[e]Percent recoveries were calculated based on the amount of viruses (total PFU) present in the sewage before the addition of coagulants as 100%.

[f]Sludge solids content was expressed as a percentage on a weight to volume basis.

[g]A dash means not done.

TABLE 4-21. Association between poliovirus type 1 and chemical sludge solids

Sludge[a] type	Experiment no.	Virus in unfractionated[b] sludge (total PFU)	Virus in sludge supernatant[c] (total PFU)	Viable unadsorbed[d] virus (%)	Solids-associated[e] virus (%)
Alum	1	1.2×10^7	3.6×10^5	3.0	97.0
	2	4.3×10^7	1.5×10^5	0.3	99.7
	3	1.1×10^7	5.0×10^4	0.5	99.5
Ferric chloride	1	4.7×10^6	6.5×10^4	1.4	98.6
	2	4.4×10^6	1.2×10^5	2.7	97.3
	3	5.2×10^6	7.0×10^4	1.3	98.7
Lime	1	7.5×10^4	0	0	100.0
	2	0	0	0	0

[a]The chemical sludges were precipitated from virus-seeded, raw sewage. The methods used to produce these sludges and to determine the amount of viruses present in the sludges are described in Table 4-20.

[b]The sludge solids were not separated prior to assaying.

[c]The sludge was clarified by centrifugation at 1400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[d]The "viable unadsorbed virus (%)" values were calculated as shown in the Materials and Methods section.

[e]The "solids-associated virus (%)" values were estimated as shown in the Materials and Methods section.

TABLE 4-22. Retention of poliovirus type 1 by packed columns of Red Bay sandy loam subsoil following the application of chemical sludges and the subsequent leaching with rain water

Sludge type	Total no. of pore volumes[a] eluted	Sludge[b] volume applied (ml)	Sludge solids applied (g)	Total no. of poliovirus, PFU		Range of conductivity values for pore volumes collected (μmho/cm at 25°C)	Range of pH values for pore volumes collected
				Contained in the sludge applied	Passing through the soil[c]		
Alum	Column 1						
	5.0	15	-	9.0×10^6	0	43 - 142	5.4 - 5.5
	Column 2						
	10.0	43	0.1	3.7×10^7	0	30 - 240	5.0 - 5.6
Ferric chloride	Column 1						
	10.0	22	0.1	3.3×10^6	0	32 - 175	5.5 - 5.7
	Column 2						
	9.0	25	0.1	3.3×10^6	0	31 - 187	4.8 - 5.1
Lime	Column 1						
	2.0	24	-	6.0×10^4	0	95 - 130	5.4 - 5.6
	Column 2						
	10.0	32	0.3	0	0	27 - 145	5.6 - 6.9

[a]One pore volume for these columns equals 225 ml. The laboratory packed columns were 29 cm in length and 4.8 cm internal diameter; the columns were filled only 27 cm with soil (2 cm left on top for packed sludge), and were conditioned with 2 pore volumes of rain water. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3).

[b]The chemical sludges were precipitated from virus-seeded, raw sewage. The methods used to produce these sludges and to determine the amount of viruses present in the sludges are described in Table The sludges were applied to the soil columns, allowed to soak in, and then, worked under 2.5 cm. Elution with rain water was subsequently undertaken. The rain water was applied continuously to the columns at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.

[c]Soil leachates were collected in 1/2 pore volume fractions and assayed individually for viral infectivity. In order to detect small numbers of viruses, pore volumes 0.5 through 5.0 (and 5.5 through the final pore volume) were subsequently combined and concentrated 160-fold by membrane filtration. The concentrates were assayed for poliovirus. This concentration procedure was only performed on the leachates from the columns receiving alum (Column 2) and ferric chloride sludge.

be detected in any of the soil leachates despite the concentration of the leachates by membrane filtration (160-fold concentration).

Lime-Stabilized, Chemical Sludges

Large quantities of chemical sludge are usually produced during primary treatment and may be stabilized with the use of lime (Farrell et al. 1974). The effect of lime stabilization on the infectivity of poliovirus present in chemical sludges (alum and ferric chloride) was investigated next. The fate of poliovirus following the application of lime-stabilized, chemical sludges to soil columns was also studied.

The stabilization of chemical sludges (alum and ferric chloride sludges) with lime resulted in almost complete inactivation of poliovirus (Tables 4-23 and 4-24). It was, thus, not surprising to observe that the virus did not break through when the stabilized sludges were applied to soil columns of Red Bay sandy loam which were subsequently leached with ten pore volumes of rainwater (Table 4-25).

These results show that lime stabilization of chemical sludges can effectively inactivate viruses. Moreover, as in the case of conditioned-dewatered sludge and chemical sludges, viruses remaining in lime-stabilized, chemical sludges can be effectively retained by soils following sludge disposal on land.

Effect of Soil Bulk Density on Poliovirus Transport

The effect of soil bulk density on poliovirus transport was studied using laboratory-packed soil columns of Red Bay sandy loam subsoil. The air-dried soil was packed into 10-cm acrylic plastic columns at bulk densities of 1.45 and 1.60 g/cm^3. Poliovirus was suspended in primary

TABLE 4-23. Inactivation of poliovirus type 1 following lime stabilization of chemical sludges

Sludge[a] type	Concentration of lime[b] used (mg/l)	pH, 30 min after the addition of lime	Virus in unfractionated[c] sludge, before liming	Virus in unfractionated sludge, 30 min after liming	
			Total PFU	Total PFU	Recovery[d] (%)
Alum[e]	1389	11.3	1.1×10^7	0	0
Ferric chloride[e]	625	11.1	5.2×10^6	6.2×10^3	0.1

[a]The chemical sludges were precipitated from virus-seeded, raw sewage. The methods used to produce these chemical sludges and to determine the amount of viruses present in the sludges are described in Table 4-20.

[b]An aqueous slurry of lime (5% $Ca(OH)_2$) was added to the chemical sludges shown until a pH of 11.5 was achieved and maintained for 5 min. The final concentrations of $Ca(OH)_2$ used appear in the table above. A contact time of 30 min was allowed while mixing the suspension on a magnetic stirrer.

[c]The sludge solids were not separated prior to assaying.

[d]Percent recoveries shown were calculated based on the corresponding unfractionated sludge assay before liming as 100%.

[e]The lime-stabilized, chemical sludges were applied to columns of Red Bay sandy loam subsoil (see Table 4-25).

TABLE 4-24. Association between poliovirus type 1 and lime-stabilized, chemical sludge solids

Sludge[a] type, lime-stabilized	Virus in unfractionated[b] sludge (total PFU)	Virus in sludge supernatant[c] (total PFU)	Viable unadsorbed[d] virus (%)	Solids-associated[e] virus (%)
Alum	0	0	0	0
Ferric chloride	6.2×10^3	0	0	100.0

[a]The chemical sludges were precipitated from virus-seeded, raw sewage. The methods used to produce these sludges and to determine the amount of viruses present in the sludges are described in Table The chemical sludges were then stabilized with $Ca(OH)_2$ as described in Table 4-23.

[b]The sludge solids were not separated prior to assaying.

[c]The sludge was clarified by centrifugation at 1400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[d]The "viable unadsorbed virus (%)" values were calculated as shown in the Materials and Methods section.

[e]The "sludge solids-associated virus (%)" values were estimated as shown in the Materials and Methods section.

TABLE 4-25. Retention of poliovirus type 1 by packed columns of Red Bay sandy loam subsoil following the application of lime-stabilized, chemical sludges and the subsequent elution with rain water

Sludge type, lime-stabilized	Total no. of pore volumes[a] eluted	Sludge[b] volume applied (ml)	Sludge solids applied (g)	Total no. of poliovirus, PFU		Range of conductivity values for pore volumes collected (μmho/cm at 25°C)	Range of pH values for pore volumes collected
				Contained in the sludge applied	Eluted from the soil[c]		
Alum	10.0	12	0.2	0	0	25 - 118	5.7 - 6.9
Ferric chloride	10.0	22	0.2	4.3×10^3	0	30 - 255	5.4 - 5.7

[a]One pore volume for these columns equals 225 ml. The laboratory-packed columns were 29 cm in length and 4.8 cm internal diameter; the columns were filled only 27 cm with soil (2 cm left on top for packed sludge), and were conditioned with 2 pore volumes of rain water. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3).

[b]The chemical sludges were precipitated from virus-seeded, raw sewage. The methods used to produce these sludges and to determine the amount of viruses present in the sludges are described in Table The chemical sludges were then stabilized with $Ca(OH)_2$ as described in Table 4-23. The lime-stabilized, chemical sludges were applied to the soil columns, allowed to soak in, and then, worked under 2.5 cm. Elution with rain water was subsequently undertaken. The rain water was applied continuously to the columns at approximately 5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The rain water was collected next to the Environmental Engineering Sciences building at the University of Florida, Gainesville. See Table 4-2 for chemical characteristics of the rain water.

[c]Soil leachates were collected in 1/2 pore volume fractions and assayed individually for viral infectivity. In order to detect small numbers of viruses, pore volumes 0.5 through 5.0 (and 5.5 through 10.0) were subsequently combined and concentrated 160-fold by membrane filtration. The concentrates were assayed for poliovirus. This concentration procedure was only performed on the leachates from the column receiving lime-stabilized, ferric chloride sludge.

wastewater effluent (pH of 7.5 and conductivity of 340 μmho/cm at 25°C) and applied continuously to the soil columns at approximately 3.5 ml/min using a peristaltic pump. As shown in Figure 4-11, poliovirus moved faster and appeared in the leachates in greater numbers in columns packed at a bulk density of 1.45 g/cm^3 (i.e., columns 1 and 2) than in columns packed at a bulk density of 1.60 g/cm^3. Only column 3 at a bulk density of 1.45 g/cm^3 displayed slow movement through the soil typical of the soil columns at a bulk density of 1.60 g/cm^3 (see Figure 4-11). It appears that in the columns displaying large viral breakthroughs (i.e., columns at bulk density of 1.45 g/cm^3), the virus-seeded primary effluent followed a less circuitous path through the soil resulting in less opportunity for poliovirus adsorption to the soil particles. In spite of this, however, no significant statistical difference was found between the fractions of poliovirus eluted at the tenth pore volume in soil columns packed at the 2 different bulk densities (see Table 4-26).

FIGURE 4-11. Movement of poliovirus type 1 suspended in primary wastewater effluent through 10-cm columns of Red Bay sandy loam subsoil packed at bulk densities of 1.45 and 1.60 g/cm^3

One pore volume for the columns packed at a bulk density of 1.45 or 1.60 g/cm^3 equals 80 or 70 ml, respectively (see Table 4-5). The laboratory-packed columns were 10 cm in length and 4.8-cm internal diameter. The sample of Red Bay sandy loam subsoil used consisted mainly of the A2 and B1t horizons (see Table 4-3). The columns were conditioned with 2 pore volumes of nonseeded primary wastewater effluent. Poliovirus was then suspended in the primary wastewater effluent at the concentrations shown in the figure in parentheses and applied to the columns. All solutions were applied continuously to the columns at approximately 3.5 ml/min using a peristaltic pump (Buchler, Fort Lee, N.J.). The primary wastewater effluent sample used displayed a pH of 7.5 and a conductivity of 340 μmho/cm at 25°C. The mean pH and conductivity (ranges indicated by vertical lines) of each pore volume were calculated from the individual values obtained for the 6 columns.

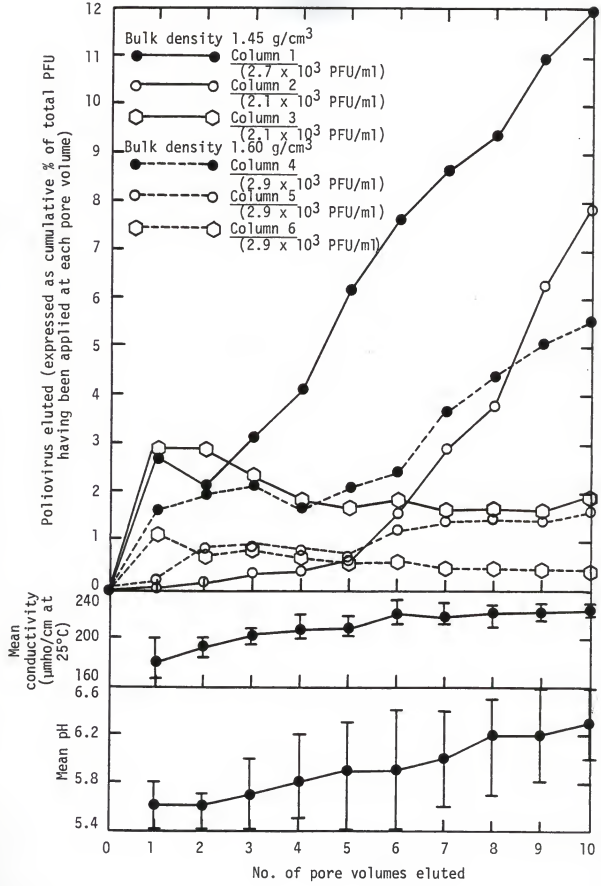

Bulk density 1.45 g/cm3
Column 1 (2.7 x 10^3 PFU/ml)
Column 2 (2.1 x 10^3 PFU/ml)
Column 3 (2.1 x 10^3 PFU/ml)
Bulk density 1.60 g/cm3
Column 4 (2.9 x 10^3 PFU/ml)
Column 5 (2.9 x 10^3 PFU/ml)
Column 6 (2.9 x 10^3 PFU/ml)
Poliovirus eluted (expressed as cumulative % of total PFU having been applied at each pore volume)
Mean conductivity (µmho/cm at 25°C)
Mean pH
No. of pore volumes eluted

TABLE 4-26. Total amount of poliovirus type 1 detected in ten pore volumes of leachate from 10-cm columns of Red Bay sandy loam subsoil packed at bulk densities of 1.45 and 1.60 g/cm^3

Bulk density[a] (g/cm^3)	Column no.	At pore volume no. 10		
		Poliovirus applied (total PFU)	Poliovirus breakthrough (%)[b]	Mean[c] poliovirus breakthrough for each bulk density (%[b] ± SE[d])
1.45	1	2.2×10^6	12	7.3 ± 2.9
	2	1.7×10^6	7.9	
	3	1.7×10^6	2.0	
1.60	4	2.0×10^6	5.6	2.6 ± 1.6
	5	2.0×10^6	1.6	
	6	2.0×10^6	0.5	

[a]Complete data and experimental procedures are shown in Figure 4-11.

[b]Expressed as cumulative percent of total PFU applied at pore volume no. 10.

[c]The two mean values shown are not significantly different at the 0.05 level when subjected to a two-tailed,t-test.

[d]Abbreviation for standard error.

CHAPTER V
RETENTION AND INACTIVATION OF ENTEROVIRUSES IN SOIL CORES TREATED WITH VIRUS-SEEDED SLUDGE AND EXPOSED TO THE NORTH-CENTRAL FLORIDA ENVIRONMENT

Introduction

Although numerous studies have been conducted to determine the survival (Bagdasaryan 1964; Derbyshire and Brown 1978; Duboise et al. 1976; Green 1976; Lefler and Kott 1974; Hurst et al. 1980a, 1980b; Moore et al. 1977; Sobsey et al. 1980a; Yeager and O'Brien 1979a, 1979b) and transport pattern (Bitton et al. 1976; Drewry and Eliassen 1968; Duboise et al. 1976; Funderburg et al. 1979; Gerba and Lance 1978; Greene 1976; Laak and McClean 1967; Lance and Gerba 1980; Lance et al. 1976; Landry et al. 1980; Lefler and Kott 1974; Robeck et al. 1962; Schaub and Sorber 1977; Scheuerman et al. 1979; Sobsey et al. 1980a; Young and Burbank 1973) of viruses following water or wastewater application to soils, few have been undertaken to assess viral persistence and transport in sludge-treated soils (see reviews by Bitton 1975, 1978, 1979b, 1980a, 1980b; Duboise et al. 1979; Foster and Engelbrecht 1973). Viruses have been found to be inactivated in sludge allowed to dry on the soil surface (Brown et al. 1980; Hurst et al. 1978; Nielsen and Lydholm 1980). Other investigators have demonstrated that, under cold winter temperatures, viruses can persist in sludge-amended soils for as long as six months (Tierney et al. 1977; Damgaard-Larsen et al. 1977). Damgaard-Larsen et al. (1977) used lysimeters to study viral

transport in sludge-amended soils and noted that viruses were completely retained by the soils under study.

In this chapter, the survival and transport of viruses in sludge-treated soils were evaluated under field conditions. Undisturbed soil cores were used and environmental parameters (i.e., soil temperature, soil moisture, and rainfall) were monitored. The protocol of sludge disposal to soil was similar to that practiced at sludge disposal sites. Virus survival and transport were monitored during three different runs, using the same soil cores.

Materials and Methods

Viruses and Viral Assays

Poliovirus type 1 (strain LSc) and echovirus type 1 (strain Farouk-prototype strain according to Wulff and Chin 1972) were used in the research reported in this chapter. Some general properties of echoviruses are shown in Table 5-1 (see Table 3-1 for general properties of polioviruses). Stocks of echovirus were prepared as described for poliovirus in Chapter III (see page 53). Viral stocks were concentrated by either ultracentrifugation or by the method developed by Farrah et al. (1978) that involved blending with trichlorotrifluoroethane (Freon 113, DuPont DeNemours Co., Wilmington, Delaware) followed by concentration on Filterite filters (Filterite Corp., Timonium, Maryland). The concentrated viruses were kept at -70°C until used. Echovirus was assayed by the plaque technique as described for poliovirus in Chapter III (see pages 53-56). Each viral count shown represents

TABLE 5-1. General properties of echoviruses

Property	Value[a]
Nucleic acid	RNA (single-stranded)
Molecular weight of nucleic acid (daltons)	2×10^6
Particle diameter (nm)	17 to 30
Morphology	Icosahedral
Stability at 4°C	Stable for 1 to 2 years
Stability at pH 3.0	Stable for 3 hours at 25°C
Stability in ether	Stable

[a]All data were obtained from Wulff and Chin (1972).

the average of triplicate counts. The numbers of viruses were expressed as plaque-forming units (PFU).

Sludges

Two sludge types were used in these experiments: aerobically digested sludge (GDA 180--see Table 3-2) sampled at the Main Street wastewater treatment plant of Gainesville, Florida, and lagooned sludge (LAG--see Table 3-2) sampled at the West Florida Agricultural Experiment Station, Jay, Florida. The lagooned sludge is a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3) from the Montclair and Main Street wastewater treatment plants of Pensacola, Florida, respectively (see Table 3-2). The mixture was kept in a lagoon at the experiment station before ultimately being disposed of on land. The sludges were collected and sludge parameters (i.e., pH and solids content) were measured as described in Chapter III (see page 56). The sludge conductivity was measured using a Beckman conductivity bridge model RC 16B2 (Beckman Instruments, Fullerton, California). The sludges used were not autoclaved or decontaminated in any other way.

Association of Seeded Viruses with Sludge Solids

Poliovirus or echovirus stock in phosphate-buffered saline (PBS) containing 2% fetal calf serum (FCS) (see Appendix for more details on the composition of this solution) was added directly to sludge at the rate of 1 ml of virus stock per 1,000 ml of sludge and while stirring the suspension using a magnetic stirrer. Magnetic stirring was continued for 10 to 60 minutes and then the association of viruses with sludge solids was determined using the procedure outlined in detail in

Chapter III(See page 62). Briefly, an aliquot of the unfractionated sludge (i.e., sludge sample without solids separated) was diluted in PBS containing 2% FCS and assayed directly for viruses by the plaque technique. This method (i.e., sludge dilution and subsequent direct assay on cell cultures) has been previously shown to be highly efficient in the recovery of poliovirus from unfractionated sludge (see Chapter III, page 62 and Table 3-3). Similar efficient recovery of echovirus from unfractionated sludge was also observed. The unfractionated sludge assay was performed in order to determine the total amount of virus present in the sludge sample. An aliquot of the sludge was subsequently centrifuged at 1,400 x g for 10 minutes at 4°C. The sludge supernatant produced was assayed for viruses. The "viable unadsorbed virus" and "sludge solids-associated virus" fractions were calculated as shown in Chapter III (see page 64). Following the initial viral assays, virus-seeded sludge was applied to soil cores as described below.

Soil

The soil used in the research reported in this chapter was a Eustis fine sand sampled at the agronomy farm, University of Florida, Gainesville. This soil was classified as a Psammentic Paleudult, sandy, siliceous, hyperthermic (Calhoun et al. 1974). Some characteristics of this soil are shown in Table 4-3. The percent organic matter in this soil was measured at less than 1% (Calhoun et al. 1974).

Fate of Viruses in Soil Cores

The survival and transport (i.e., movement or retention) of poliovirus and echovirus in soil cores treated with virus-seeded sludge was studied under natural conditions.

Undisturbed soil cores. Undisturbed soil cores (Blake 1965; Sanks et al. 1976) of Eustis fine sand were used and they were obtained by driving polyvinyl chloride pipes into the soil at the agronomy farm, University of Florida, Gainesville, as described in Chapter IV (see pages 102-103). The undisturbed soil cores were obtained in a manner that preserved the natural structure and bulk density of the soil as found in the field (see Table 4-3). The soil cores were 33 cm in length (pipes were 40 cm in length) and had an internal diameter of 5.0 cm (small soil cores) or 15.5 cm (large soil cores), and thereby consisted of the Ap and A21 horizons of the fine sand (see Table 4-3). Two small soil cores and four large soil cores were employed. A polypropylene screen (105-μm pore size) which supported the soil while allowing the free movement of water and viruses (i.e., did not adsorb viruses in soil leachates), and a spout were secured at the bottom of each small soil core. Porous ceramic cups attached to spouts, on the other hand, were installed at the bottom of the large soil cores. The porous ceramic cups used were 6.9 cm long, and had a wall thickness of 0.23 cm and a pore diameter of 1.4 to 2.1 μm (no. 2131, Soil Moisture Equipment Corp., Santa Barbara, California). These cups restricted the movement of water somewhat and consequently produced an artificial groundwater table in the bottom part of the soil during

periods of rainfall. Similarly, Robeck et al. (1962) simulated a groundwater table in a sand column that was sealed at the bottom. The capacity of the porous ceramic cups to retain viruses was evaluated as described below. All six soil cores were exposed to natural conditions outside the Environmental Engineering Sciences building at the University of Florida, Gainesville. The soil cores rested on a wooden box such that soil leachates produced during natural rainfall could be collected (see Figure 5-1). Unlike the small soil cores, the large soil cores were insulated by surrounding them with duct insulation as shown in Figure 5-1. All soil cores were treated with virus-seeded sludge as described below.

Porous ceramic cups. The retention capacity of porous ceramic cups towards viruses was evaluated using poliovirus suspended in a rain leachate from a small undisturbed core (see above) of Eustis fine sand. The rain leachate was produced in the laboratory by passing rain water continuously through the small soil core. The rain water was applied from an inverted, self-regulated, 1-liter Erlenmeyer flask set to maintain a 2.5-cm hydraulic head on the soil core (Sanks et al. 1976). Leachate from the soil core (400 ml) was seeded with poliovirus. The virus-seeded leachate was then divided into two fractions of 200 ml and each fraction was passed through a sterile ceramic cup (no. 2131--see characteristics above) with the use of a vacuum pump at a flow rate of approximately 2 ml hr^{-1} cm^{-2}. The concentration of poliovirus in rain leachate was determined before and after passage through the porous ceramic cups in order to claculate the percent retention of the virus.

FIGURE 5-1. Photograph of the soil cores of Eustis fine sand used in this study

Details on the procedures used to prepare these soil cores appear on pages 183 to 184. Four large soil cores (i.e., LC1 through LC4) and two small soil cores (i.e., SC1 and SC2) were employed as seen in the photograph.

As shown in Table 5-2, 38.7% (mean for the two ceramic cups) of poliovirus suspended in rain leachate was lost (presumably retained by ceramic cups) during passage through the porous ceramic cups. Other investigators have found substantially greater retention of poliovirus type 1 by similar porous ceramic cups (i.e., 75% to 99.7%) when the virus was seeded in dechlorinated tapwater or in unchlorinated activated sludge effluent (Sobsey 1976; Wang et al. 1980b). These solutions displayed greater conductivity values [e.g., 580 μmho/cm for tapwater and 787 μmho/cm for activated sludge effluent (Wang et al. 1980b)] than that found for the rain leachate (i.e., 18 μmho/cm--see Table 5-2) employed in this study. Consequently, the abundantly present salts in tapwater and activated sludge effluent promoted greater viral adsorption to the ceramic material. Wang et al. (1980b) also demonstrated that echovirus type 1 (strain V239--isolated from groundwater) seeded in tapwater or activated sludge effluent was retained (i.e., 30% to 86%) by ceramic cups but to a lesser degree than observed for poliovirus type 1. Since the strain of echovirus type 1 used was previously shown to adsorb poorly to soil, it is likely that this virus did not adsorb efficiently to the ceramic material either (Wang et al. 1980b). In addition to viruses, fecal coliforms in water have also been reported to be retained by ceramic cups (Dazzo and Rothwell 1974). It is worth noting that the rain leachate used herein to evaluate viral retention by ceramic cups closely approximates the chemical composition of soil water actually passing the ceramic cups installed at the bottom of the large soil cores (see above). As

TABLE 5-2. Retention of poliovirus type 1 by porous ceramic cups

Influent[a] virus concentration (PFU/ml)	Effluent[b] virus concentration (PFU/ml)	Virus retained by ceramic cup[c] (%)
2.7×10^4	1.8×10^4	33.3
2.5×10^4	1.4×10^4	44.0
	Mean:	38.7

[a]Poliovirus was suspended in a rain leachate (conductivity and pH equal to 18 μmho/cm at 25°C and 6.0, respectively) from an undisturbed core of Eustis fine sand, and passed through sterile, porous, ceramic cups with the use of a vacuum pump at a flow rate of approx. 2 ml hr^{-1} cm^{-2}.

[b]Concentration of virus after passage through a porous ceramic cup.

[c]The porous ceramic cups used were 6.9 cm long and had a wall thickness of 0.23 cm (no. 2131, Soil Moisture Equipment Corp., Santa Barbara, California). The cups used had a pore diameter of 1.4 to 2.1 μm.

indicated by the data in Table 5-2, substantial retention of poliovirus by the ceramic cups at the bottom of the large soil cores is quite likely. In light of the research by Wang et al. (1980b), a significant fraction of echovirus type 1 would probably also be lost during passage through the ceramic cups on the large soil cores. Due to the possible viral loss, the entire leachate volume from the large soil cores must be evaluated for the presence of viruses.

Application of virus-seeded sludge to soil cores. Sludge seeded with poliovirus was applied to two large soil cores (i.e., LC3 and LC4--see Figure 5-1) in October 1977, June 1978, and October 1978. Poliovirus-seeded sludge was also applied to two small soil cores (i.e., SC1 and SC2--see Figure 5-1) in June 1978 and to one small core (i.e., SC2) in October 1978. Sludge seeded with echovirus was applied to two other large soil cores (i.e., LC1 and LC2--see Figure 5-1) and to one small core (i.e., SC1 which had received poliovirus-seeded sludge in June 1978) in October 1978. Each soil core was treated with 2.5 cm of virus-seeded sludge which is equivalent to a liquid application rate of 254 m^3/ha. The applied sludge was allowed to soak in and dry on top of the soil for one to four days. During this period, the drying sludge solids on the soil surface of the large soil cores were monitored for the presence of viruses, as described below. Following the drying period, the sludge resting on the soil surface was mixed with the top 2.5 cm of soil. The top 2.5 cm of soil in the large soil cores was then monitored for the presence of seeded viruses as described below. Soil monitoring was continued

until viruses could no longer be detected. The small soil cores were not used to study viral survival in sludge-treated soil but rather were used to evaluate viral transport as described before.

Leachates from soil cores. Leachates from all sludge-treated soil cores were collected during natural rainfall as shown in Figure 5-1. In June and July 1978, the small soil cores (i.e., SC1 and SC2--see Figure 5-1) treated with poliovirus-seeded sludge were periodically leached with rain water (see Table 4-2 for chemical characteristics) applied from inverted, self-regulated, 1-liter Erlenmeyer flasks set to maintain a 2.5-cm hydraulic head on the cores (Sanks et al. 1976). The artificial leaching consisted of applying the rain water at a flow rate of approximately 3.9 ml/min until approximately one pore volume (234 ml for small soil cores) of leachate was collected. All leachate samples (i.e., natural or artificial) were promptly taken into the laboratory where their volumes were accurately measured [reported volume in ml, cm, and pore volumes (calculated according to equations 4-1 and 4-2)]. The pH and conductivity of each leachate sample were then measured using the procedures described above for sludges. Finally, each leachate sample was concentrated by membrane filtration and assayed for seeded viruses as described below.

Virus Recovery Procedures

Sludge. Samples of drying sludge were obtained from the surface of the large soil cores (one sample per soil core per sampling date). THe solids content of each sludge sample was then determined as described below. Seeded viruses (i.e., poliovirus or echovirus) were

eluted from 1-g (wet weight) samples of drying sludge using the glycine procedure developed by Hurst et al. (1978). This method consisted of mixing each 1-g sludge sample with 5 ml of 0.05 M glycine buffer, pH 11.5. If necessary, the pH of the mixture was adjusted to between 10.5 and 11.0 by the addition of 1M glycine buffer, pH 11.5. The samples were vigorously vortexed for one minute and centrifuged at 14,000 x g for 5 minutes at 4°C (all centrifugation was performed using a Sorvall RC5-B centrifuge, Ivan Sorvall Inc., Norwalk, Connecticut). The supernatants (i.e., the sludge solids eluates) were recovered, adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for eluted viruses as described above. Further viral concentration was not required. The entire procedure described above was performed in less than 10 minutes. Thus, poliovirus and echovirus were subjected to the high pH of 10.5 to 11.0 for no more than 10 minutes. Both Hurst et al. (1978) and Sobsey et al. (1980) observed no appreciable inactivation in 10 minutes of poliovirus type 1 (LSc) seeded in 0.05 M glycine buffer, pH 10.5 to 11.0. Therefore, it is believed that there was no significant inactivation of poliovirus or echovirus during the elution of sludge solids. The numbers of viruses recovered were expressed as PFU per g dry weight of sludge.

Soil. Soil samples were obtained from the top 2.5 cm of soil in the large soil cores (one sample per soil core per sampling date). The moisture content of each soil sample was then determined as described below. Seeded viruses (i.e., poliovirus or echovirus) were eluted from 10-g (wet weight) samples of soil using the procedure

described by Bitton et al. (1979a). This method consisted of mixing each 10-g soil sample with 20 ml of 0.5% (wt./vol.) isoelectric casein (Difco Laboratories, Detroit, Michigan), pH 9.0. If necessary, the pH of the mixture was adjusted to between 9.0 and 9.2 by the addition of 5 M Trizma base (Sigma Chemical Co., St. Louis, Missouri). The samples were vigorously vortexed for 30 seconds and then shaken on a rotating shaker for 15 minutes. The samples were subsequently centrifuged at 1,400 x g for 4 minutes at 4°C. The supernatants (i.e., the soil eluates) were recovered and immediately adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0. Viruses in the soil eluates were concentrated by organic flocculation (Katzenelson et al. 1976b) as follows. The eluates were adjusted to pH 4.4 by the addition of 1 M glycine buffer, pH 2.0. The floc produced were pelleted by centrifugation at 160 x g for 1 minute at 4°C. The supernatants were discarded. The pellets were mixed with 2 ml of 0.15 M Na_2HPO_4, pH 9.0. The mixtures were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 11.5, and then magnetically stirred until the pellets were completely resolubilized. The samples were subsequently centrifuged at 14,000 x g for 10 minutes at 4°C. The supernatants were adjusted to neutral pH (i.e., if necessary), adjusted to a final concentration of 2% FCS and assaysed for eluted viruses as described above. The numbers of viruses recovered were expressed as PFU per g dry weight of soil.

Leachates. Leachate samples were concentrated by membrane filtration (Farrah et al. 1976; Hill et al. 1971; Shuval and Katzenelson

1972; Sobsey et al. 1973; Sobsey et al. 1980b) as follows. Each leachate sample was adjusted to pH 3.5 by the addition of 1 M glycine buffer, pH 2.0, and adjusted to a final concentration of 0.0005 M aluminum chloride. The treated water was then passed through a series of 3.0-, 0.45-, and 0.25-µm Filterite filters in a 47-mm holder. Adsorbed viruses were eluted from the filters with 7 ml of PBS containing 10% FCS, pH 9.0. The filter eluate was adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for seeded viruses as described above. The quantity of poliovirus or echovirus detected in each leachate sample was expressed as total PFU and as a percentage of the amount of virus applied to the soil (i.e., cumulative percent of the total viral PFU applied was calculated according to Equation 4-4).

Measurement of Environmental Parameters

The soil temperature, soil moisture, and rainfall were monitored. The soil temperature was monitored every hour using thermocouples placed at the soil surface and at depths of 2.5, 10, and 20 cm on one of the large soil cores as shown on Figure 5-2. The thermocouples were connected to an Esterline Angus Key Programmable Data Acquisition System (Model PD-2064, Esterline Angus Instrument Corporation, Indianapolis, Indiana) which printed voltage (millivolts) at each thermocouple every hour. The voltages measured were later converted to temperature readings with the use of a computer. The soil moisture was monitored only when a sample of soil was obtained for viral assay. Soil moisture content was determined gravimetrically

FIGURE 5-2. Photograph of a large soil core of Eustis fine sand (LC4) shown with thermocouples placed at the soil surface and at depths of 2.5, 10, and 20 cm

The thermocouples were used to monitor the soil temperature. Details on the procedures used to prepare this soil core appear on pages 183 to 184 and on pages 193 to 196.

on a wet-weight basis by drying in an oven at 105°C for 24 hours a measured weight of wet soil from the top inch of the soil cores and was expressed as a percentage as follows:

$$\text{Soil moisture (\%)} = \frac{\text{wet soil weight (g)} - \text{dry soil weight (g)}}{\text{wet soil weight (g)}} \times 100 \qquad (5\text{-}1)$$

Unfortunately, soil moisture could not be measured more frequently because it could not be automated. The rainfall was measured next to the soil cores with a farm rain gauge (model no. 510, Science Associates, Inc., Princeton, N.J.) attached to the wooden box as seen in Figure 5-1. The rainfall was measured after each rain event in centimeters. Chemical parameters for the rainfall at the experimental site are presented in Table 4-2. In the first survival experiment that began on 7 October 1977, measurement of some environmental parameters (i.e., temperature and rainfall) could not be carried out at the experimental site due to the lack of the necessary equipment. In this case, the data from the weather station of the Department of Agronomy, University of Florida, were used. This station is approximately one mile from the experimental site.

Results and Discussion

In the previous chapter (i.e., Chapter IV), virus transport through soils was evaluated under controlled laboratory conditions. It appeared necessary, however, to study virus transport and survival under more natural conditions. Undisturbed soil cores were used to assess viral transport and survival under field conditions. In these

experiments, environmental parameters (i.e., temperature, soil moisture, and rainfall) were monitored. The protocol of sludge disposal to soil was similar to that practiced at sludge disposal sites. Virus survival and transport were monitored during three different runs, using the same cores. The survival monitoring was terminated when viruses were not detectable in soil samples.

Association between Seeded Enteroviruses and Sludge Solids

Prior to studying virus transport through soil cores, it was necessary to assess the extent of virus association with sludge solids. Poliovirus was added to aerobically digested sludge and to lagooned sludge (2/3 anaerobic and 1/3 aerobic sludge), while echovirus was added to lagooned sludge only. Following magnetic stirring for 10 to 60 minutes, the fraction of sludge solids-associated virus was determined. As shown in Table 5-3, more than 90% of poliovirus was found associated with sludge solids (aerobic or lagooned sludge). On the other hand, only 20.7% of seeded echovirus was observed to be associated with lagooned sludge solids (see Table 5-3). The virus-seeded sludge was then applied to the undisturbed soils cores. The association between viruses and sludge solids may be instrumental in virus retention during sludge application to land.

First Survival Experiment (7 October 1977-12 October 1977)

During this period, the soil temperature was not monitored. However, air temperature data were obtained from the weather station of the Department of Agronomy, University of Florida (Figure 5-3). It

TABLE 5-3. Association between seeded enteroviruses and sludge solids

Virus[a] used	Sludge used	Sludge parameters			Virus in unfractionated[b] sludge (total PFU)	Virus in sludge supernatant[c] (total PFU)	Viable unadsorbed virus (%)	Solids-associated virus (%)	Unfractionated sludge applied to soil cores[d] as described in
		pH	Conductivity (μmho/cm at 25°C)	Solids content (%)					
Poliovirus type 1	Aerobic[e]	5.0	--	1.3	3.9×10^8	2.3×10^6	0.6	99.4	Table 5-4
	Lagoon[f]	6.9	3500	2.9	6.1×10^8	5.4×10^7	8.9	91.1	Table 5-5
	Lagoon	7.0	1525	7.0	8.6×10^8	4.1×10^7	4.8	95.2	Table 5-8
Echovirus type 1	Lagoon	7.0	1525	7.0	2.9×10^6	2.3×10^6	79.3	20.7	Table 5-8

[a]Virus was added to sludge while stirring the suspension using a magnetic stirrer. Magnetic stirring was continued for 10 to 60 min and then an aliquot of the virus-seeded sludge was obtained for viral assay.

[b]The sludge solids were not separated prior to assaying. The values shown represent the total virus PFU contained in 479 ml of sludge.

[c]The sludge was clarified by centrifugation at 1,400 x g for 10 min at 4°C and the supernatant was subsequently assayed.

[d]Undisturbed large soil cores received 2.5 cm or 479 ml of virus-seeded sludge.

[e]Aerobically digested sludge (GDA180--see Table 3-2) was sampled at the Main Street wastewater treatment plant of Gainesville, Florida.

[f]Lagoon sludge (LAG--see Table 3-2) is a mixture of aerobically digested sludge (1/3) and anaerobically digested sludge (2/3). The mixture was kept in a lagoon at the West Florida Agricultural Experiment Station (Jay, Florida) before ultimately being disposed of on land.

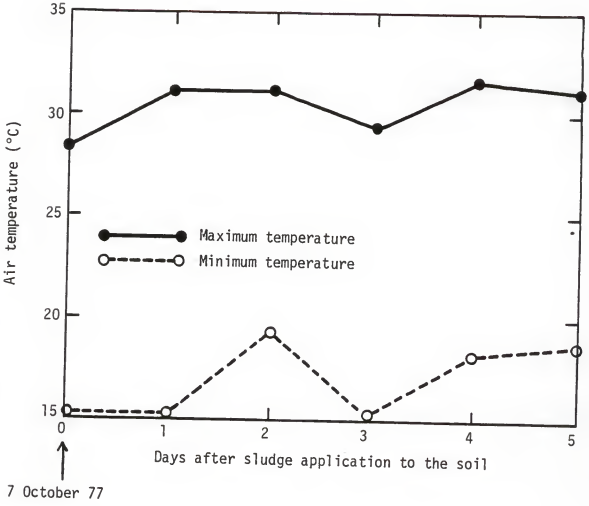


FIGURE 5-3. Daily air temperature (5 ft above ground) for the duration of the survival experiment that began 7 October 1977

Data were collected at the weather station of the Department of Agronomy, University of Florida, Gainesville. This station is approximately 1 mile from the experimental site (i.e., next to the Environmental Engineering Sciences building, University of Florida, Gainesville). The mean, maximum temperature for the period was 30.5°C and the mean, minimum temperature for the period was 16.9°C.

is seen that air temperature was as high as 31°C and as low as 15°C. The survival of poliovirus, under natural conditions, following suspension in aerobically digested sludge (3.9×10^8 total PFU or 6.3×10^7 PFU/g dry weight of sludge) and subsequent application to soil cores is shown in Table 5-4. No virus could be recovered in the soil samples after three days. The sludge was left on top of the soil for three days (i.e., large soil core no. 4--LC4), and mixed thereafter with the top 2.5 cm of soil. During that time period, there was more than a four $\log_{10}$ reduction in virus numbers in the drying sludge (see LC4--Table 5-4). It is worth noting that during this first experiment, the rainfall was low (0.23 cm after five days--see Table 5-4) and the sludge solids increased from 1.3% to 38%. Dessication was probably the major factor which caused the rapid decline of poliovirus in the drying sludge and in the soil.

Second Survival Experiment
(2 June 1978-24 August 1978)

The second survival experiment was initiated in the summer when the weather is generally warm and wet in the Gainesville area. Temperature data were collected with thermocouples placed at the surface of the soil, and at the 2.5-, 10-, and 20-cm depths. Data analysis showed that there was no significant difference between soil temperature readings at these different depths. Therefore, only the soil temperature at the 2.5-cm depth is shown in Figure 5-4. The average temperature ranged from 23.5°C to 29°C during a 35-day period beginning

TABLE 5-4. Survival of poliovirus type 1 following suspension in liquid sludge and subsequent application to large soil cores of Eustis fine sand exposed to natural conditions (7 October 1977-12 October 1977)

Sampling date (1977)	Days after the beginning of experiment	Cumulative rainfall[a] (cm)	Sludge[b] solids content (%, wt/wt)	Soil moisture (%, wt/wt)	No. of viruses (PFU/g dry weight of sludge or soil)	
					LC3	LC4
LIQUID SLUDGE SAMPLE						
07 Oct.	0	0	1.3[c]	--[d]	6.3×10^7	6.3×10^7
DRYING SLUDGE SAMPLE						
10 Oct.	3	0.08	38.0	--	--	1.0×10^3
Sludge mixed with the top 2.5 cm of soil on day 1 (LC3) or on day 3 (LC4)						
SOIL SAMPLES (top 2.5 cm)						
08 Oct.	1	0	--	--	4.4×10^4	--
10 Oct.	3	0.08	--	11.3	2.2×10^3	4.9×10^1
12 Oct.	5	0.23	--	--	0	0

[a]Rainfall data were collected at the weather station of the Department of Agronomy, University of Florida, Gainesville. This station is approximately 1 mile from the experimental site (i.e., next to the Environmental Engineering Sciences building, University of Florida, Gainesville).

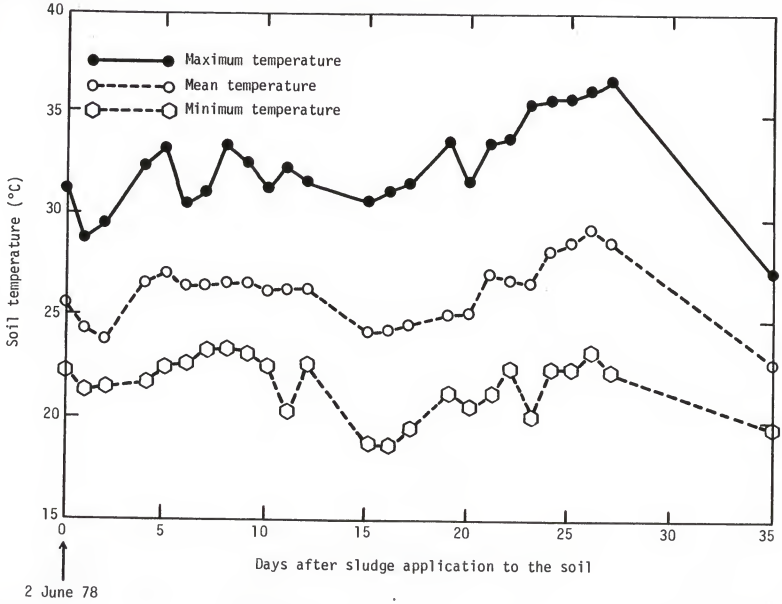
[b]One inch or 2.5 cm (254 m^3/ha) of aerobically digested sludge (see Table 5-3 for sludge characteristics) seeded with a total of 3.9×10^8 PFU of poliovirus (or 6.3×10^7 PFU/g dry weight of sludge--see Table 5-3) was applied to large soil cores of Eustis fine sand (LC3 and LC4--see Figure 5-1). The large soil cores were 33 cm in length and 15.5 cm internal diameter; they consisted of the Ap and A21 horizons of the Eustis fine sand (see Table 4-3). The seeded sludge was allowed to soak in and dry on top of the soil for 1 day or 3 days before being mixed with the top 2.5 cm of soil. The drying sludge solids and the soil were monitored for the presence of seeded viruses as detailed on pages 192 to 194.

[c]In the case of the liquid sludge, sludge solids content was expressed as a percentage on a weight-to-volume basis.

[d]A dash means not done or not applicable.

FIGURE 5-4. Daily soil temperature (2.5 cm below the soil surface of a large core of Eustis fine sand) for the duration of the survival experiment that began 2 June 1978

Data were measured at the experimental site (i.e., next to the Environmental Engineering Sciences building, University of Florida, Gainesville) using a thermocouple placed at the 2.5-cm depth in a large (33 cm in length and 15.5 cm internal diameter) undisturbed soil core of Eustis fine sand (see Table 4-3 ; consists of the Ap and A21 horizons of this soil). The soil temperature was monitored every hour at the 2.5-cm depth, as well as at the surface, 10-cm depth, and 20-cm depth. From Table 4-3 , it can be seen that all temperature readings were made in the Ap horizon of the soil. No significant difference was found between temperature readings at the surface, 2.5-cm depth, 10-cm depth, and 20-cm depth. Therefore, only the soil temperature at the 2.5-cm depth is reported.


Maximum temperature
Mean temperature
Minimum temperature
Soil temperature (°C)
40
35
30
25
20
15
0
5
10
15
20
25
30
35
Days after sludge application to the soil
2 June 78

on 2 June 1978. Figure 5-5 shows the hourly soil temperature profile on 17 June 1978. A minimum in soil temperature was observed at 6 a.m. and a maximum at 2 p.m.

With regards to rainfall, the study period was very wet with 13.63 cm of cumulative rainfall measured from 2 June through 7 July 1978 (see Table 5-5). Poliovirus survival was monitored in two soil cores which had been treated with virus-seeded sludge (see Table 5-5). In contrast to the first survival experiment (see Table 5-4), there was no drastic decline in virus numbers in the drying sludge prior to the sludge being mixed with the top 2.5 cm of soil. Soil monitoring revealed that poliovirus could be detected for up to 35 days in both soil cores. It is difficult to correlate virus survival with soil moisture since this parameter was not continuously monitored. Heavy rainfall, however, did not allow the soil (or sludge on the soil surface) to dry for an extended period of time and this probably contributed to longer virus survival (see Table 5-5).

Monitoring of soil leachates from 5 June to 24 August 1978 did not reveal any virus, despite their concentration by membrane filtration (see Table 5-6). Although 51 cm of rain fell during the study period, this represented only 0.5 to 0.7 pore volume. This is the reason why we conducted parallel studies with smaller cores (5 cm i.d. instead of 15.5 cm i.d.) which were also exposed to natural conditions, and treated with virus-seeded sludge and then leached with rainwater (the experimental leaching was continued until approximately one pore volume of leachate was collected) in addition to natural rainfall. In these core studies, some virus breakthrough was observed, but this

FIGURE 5-5. Hourly soil temperature (2.5 cm below the soil surface of a large core of Eustis fine sand) profile for 17 June 1978

Soil temperature was measured at the experimental site (i.e., next to the Environmental Engineering Sciences building, University of Florida, Gainesville) using a thermocouple placed at the 2.5-cm depth in a large soil core of Eustis fine sand (LC4--see Figures 5-1 and 5-2). The soil temperature was monitored every hour at the 2.5-cm depth, as well as at the surface, 10-cm depth, and 20-cm depth. From Table 4-3, it can be seen that all temperature readings were made in the Ap horizon of the soil. No significant difference was found between temperature readings at the surface, 2.5-cm depth, 10-cm depth, and 20-cm depth. Therefore, only the soil temperature at the 2.5-cm depth is reported. The average temperature for the day was 24.2°C. The maximum temperature was 30.8°C and it occurred at 2 P.M. The minimum temperature was 18.9°C and it occurred at 6 A.M.

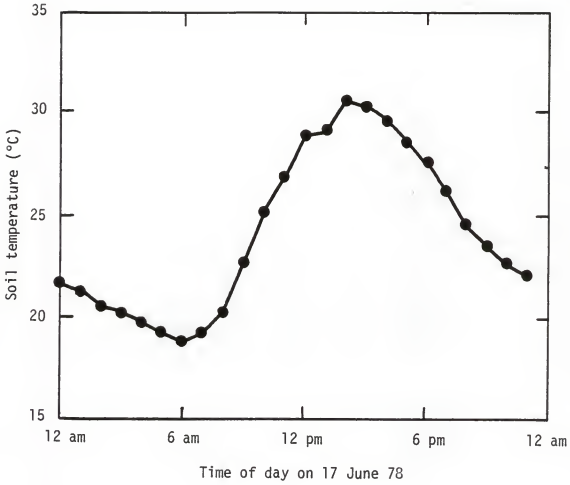

35
30
25
20
15
Soil temperature (°C)
12 am
6 am
12 pm
6 pm
12 am
Time of day on 17 June 78

TABLE 5-5. Survival of poliovirus type 1 following suspension in liquid sludge and subsequent application to large soil cores of Eustis fine sand exposed to natural conditions (2 June 1978-7 July 1978)

Sampling date (1978)	Days after the beginning of experiment	Cumulative rainfall[a] (cm)	Sludge[b] solids content (%, wt/wt)	Soil moisture (%, wt/wt)	No. of viruses (PFU/g dry weight of sludge or soil)	
					LC3	LC4
LIQUID SLUDGE SAMPLE						
02 June	0	0	2.9[c]	--[d]	4.4×10^7	4.4×10^7
DRYING SLUDGE SAMPLES						
03 June	1	1.80	23.1	--	2.5×10^6	4.9×10^6
06 June	4	6.03	--	--	1.0×10^6	5.0×10^5
Sludge mixed with the top 2.5 cm of soil on day 4						
SOIL SAMPLES (top 2.5 cm)						
06 June	4	6.03	--	14.7	3.3×10^4	4.1×10^4
07 June	5	7.03	--	0.9	8.7×10^4	7.4×10^4
09 June	7	7.28	--	9.9	2.5×10^3	2.4×10^3

23 June	21	10.45	--	0.2	4.7	17.1
07 July	35	13.63	--	0.2	1.3	0.8

[a]Rainfall data were measured at the experimental site as described on page 196.

[b]One inch or 2.5 cm (254 m^3/ha) of lagooned sludge (see Table 5-3 for sludge characteristics) seeded with a total of 6.1 x 10^8 PFU of poliovirus (or 4.4 x 10^7 PFU/g dry weight of sludge--see Table 5-3) was applied to large soil cores of Eustis fine sand (LC3 and LC4--see Figure 5-1). The large soil cores were 33 cm in length and 15.5 cm internal diameter; they consisted of the Ap and A21 horizons of the Eustis fine sand (see Table 4-3). The seeded sludge was allowed to soak in and dry on top of the soil for 4 days before being mixed with the top 2.5 cm of soil. The drying sludge solids and the soil were monitored for the presence of seeded viruses as detailed on pages 192 to 194. In addition to the large soil cores, the virus-seeded sludge was also similarly applied to two small soil cores (SC1 and SC2--see Figure 5-1) for the purpose of evaluating viral transport only. Leachates from all soil cores were monitored for viruses as shown in Tables 5-6 and 5-7.

[c]In the case of the liquid sludge, sludge solids content was expressed as a percentage on a weight-to-volume basis.

[d]A dash means not done or not applicable.

TABLE 5-6. Analysis for the presence of poliovirus type 1 (Sabin) in soil leachates collected after natural rainfall from large soil cores of Eustis fine sand which had been treated with 2.5 cm of seeded liquid sludge (2 June 1978-24 August 1978)

Dates of leachates collected (1978)	Cumulative rainfall (cm)	Soil core[a] number	Cumulative leachate volume (ml)	Cumulative number of pore volumes[b]	Cumulative virus breakthrough (total PFU)	Cumulative percent of total virus applied	Range of conductivity values of leachates collected (μmho/cm at 25°C)	Range of pH values of leachates collected
05 June-24 Aug.	51.05	C1	1544 (8.2)[c]	0.7	0	0	114-1200	6.3-7.8
05 June-24 Aug.	51.05	C2	1135 (6.0)	0.5	0	0	106-1360	5.7-7.2

[a]One inch (or 2.5 cm) of lagooned sludge seeded with a total of 6.1 x 10^8 PFU of poliovirus was applied on top of soil cores. The soil cores were exposed to natural conditions.

[b]One pore volume fore the large soil cores equals 2178 ml.

[c]Values in parentheses represent the number of centimeters of cumulative leachate volume.

represented only 0.0006% of the total viral input (see Table 5-7). These data show that some virus breakthrough can be achieved, under saturated flow, in the small cores. This had also been demonstrated in laboratory studies (see Figure 4-9). Under unsaturated flow in the large cores, however, no such breakthrough occurred (see Table 5-6).

Third Survival Experiment
(11 October 1978-20 January 1979)

From the results of the first two survival experiments, it became apparent that with regard to transport pattern, poliovirus type 1 (LSc) would not be the ideal model virus since it has a high affinity for sludge solids (see Table 5-3) and is subsequently immobilized, along with the sludge solids, at the top of the soil profile. A virus with less affinity for sludge solids would perhaps be more suitable for transport studies. Goyal and Gerba (1979) previously found that echovirus type 1 (Farouk) poorly adsorbed to soil when compared to poliovirus type 1. The association between lagooned sludge solids, and poliovirus type 1 (LSc) and echovirus type 1 (Farouk) was, therefore, investigated (Table 5-3). As presented above (see Table 5-3), echovirus was less adsorbed (20.7%)to sludge solids than poliovirus (95.2%). Lagooned sludge was, thus, seeded with either of these two enteroviruses and then applied to soil cores on 11 October 1979. Viral presence in the soil and leachates was monitored for 21 and 101 days, respectively.

During the study period, the average soil temperature, as monitored with thermocouples placed in a soil core (LC4--see Figure 5-2), ranged from 18°C to 27°C (Figure 5-6). With regard to rainfall, only

TABLE 5-7. Analysis for the presence of poliovirus type 1 (Sabin) in soil leachates collected after natural rainfall and after experimental leaching with rain water from small soil cores of Eustis fine sand which had been treated with 2.5 cm of seeded liquid sludge (2 June 1978-24 August 1978)

Small soil core[a] number	Date of leachate collected (1978)	Cumulative rainfall (cm)	Leachate volume (cm)	Cumulative leachate volume (ml)	Virus breakthrough (total PFU)	Cumulative percent of total virus applied	Conductivity of soil leachate collected (μmho/cm at 25°C)	pH of soil leachate collected
SC1	06 Jun.	6.03	16	16 (0.1)[b]	0	0	--	5.5
	06 Jun. (L.R.)[c]	24.88	317	333 (1.4)	3.9×10^2	0.0006	720	6.0
	08 Jun.	26.13	19	352 (1.5)	6.7	0.0006	--	7.2
	13 Jun.	28.88	46	398 (1.7)	0	0.0006	100	7.1
	23 Jun. (L.R.)	67.52	360	758 (3.2)	0	0.0006	430	5.8
	10 Jul.	74.25	80	838 (3.6)	0	0.0006	151	6.9
	10 Jul. (L.R.)	89.54	320	1158 (4.9)	0	0.0006	310	6.4
	13 Jul.	91.06	20	1178 (5.0)	0	0.0006	425	6.8
	03 Aug.	112.44	145	1323 (5.7)	0	0.0006	84	6.5
	24 Aug.	123.41	145	1468 (6.3) (74.8)[d]	0	0.0006	69	6.7

SC2	06 Jun.	6.03	40	40 (0.2)	0	0	770	6.8
	06 Jun. (L.R.)	22.34	282	322 (1.4)	3.9×10^2	0.0006	370	6.5
	08 Jun.	23.59	21	343 (1.5)	1.0×10^1	0.0006	--	7.3
	13 Jun.	26.34	54	397 (1.7)	0	0.0006	118	7.3
	23 Jun. (L.R.)	58.61	365	762 (3.3)	0	0.0006	480	6.3
	10 Jul.	65.34	68	830 (3.5)	0	0.0006	148	7.0
	10 Jul. (L.R.)	81.90	340	1170 (5.0)	0	0.0006	160	6.7
	13 Jul.	83.42	19	1189 (5.1)	0	0.0006	462	7.2
	03 Aug.	104.80	140	1329 (5.7)	0	0.0006	82	6.6
	24 Aug.	115.77	160	1489 (6.4) (75.9)[d]	0	0.0006	80	6.9

[a]One inch (or 2.5 cm) of lagooned sludge seeded with a total of 6.6×10^7 PFU of poliovirus was applied on top of soil cores. The soil cores were exposed to natural conditions.

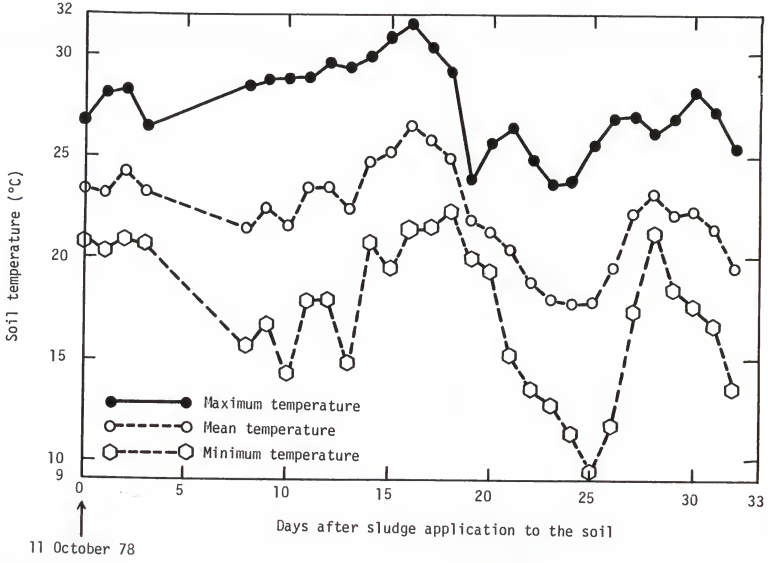
[b]Values in parentheses represent the cumulative number of pore volumes eluted. One pore volume for the small soil cores equals 234 ml.

[c]The small soil cores were priodically leached with rain water applied from inverted, self-regulated, 1-liter Erlenmeyer flasks set to maintain a 2.5-cm hydraulic head on the columns. The experimental leaching was continued until approximately one pore volume of leachate was collected and these leachates are designated L.R. (or leaching with rain).

[d]Values in the second parentheses represent the number of centimeters of cumulative leachate volume.

FIGURE 5-6. Daily soil temperature (2.5 cm below the soil surface of a large core of Eustis fine sand) for the duration of the survival experiment that began 11 October 1978

Soil temperature was measured at the experimental site (i.e., next to the Environmental Engineering Sciences building, University of Florida, Gainesville) using a thermocouple placed at the 2.5-cm depth in a large soil core of Eustis fine sand (LC4--see Figures 5-1 and 5-2). The soil temperature was monitored every hour at the 2.5-cm depth, as well as at the surface, 10-cm depth, and 20-cm depth. From Table 4-3, it can be seen that all temperature readings were made in the Ap horizon of the soil. No significant difference was found between temperature readings at the surface, 2.5-cm depth, 10-cm depth, and 20-cm depth. Therefore, only the soil temperature at the 2.5-cm depth is reported.


Soil temperature (°C)
32
30
25
20
15
10
9
Maximum temperature
Mean temperature
Minimum temperature
0
5
10
15
20
25
30
33
Days after sludge application to the soil
11 October 78

0.13 cm of rain fell on 11 October 1978 to 1 November 1978 (see Table 5-8). This was the period during which virus survival was monitored. Both poliovirus and echovirus were not detectable in soil after eight days of exposure to natural conditions in the dry fall season (see Table 5-8). The two enteroviruses were completely inactivated sometime between the eighth and the 21st day (see Table 5-8). Soil leachates were also monitored and a summary of the data is displayed in Table 5-9. Neither poliovirus nor echovirus was detected in the leachates from all the soil cores (see Table 5-9).

It appears from these studies that, under conditions prevailing in North-Central Florida, enteroviruses are rapidly inactivated during sludge application to soils. Their inactivation in the soil appears to be affected more by desiccation than by soil temperature. Under ideal conditions (warm and dry), a rapid decline of virus was observed in the sludge drying on top of the soil and in the top 2.5 cm of soil. Other investigators have shown that virus survival in sludge-treated soils is prolonged by low temperatures (Damgaard-Larsen et al. 1977; Nielsen and Lydholm 1980). Soil leachates collected after natural rainfall were negative for both poliovirus and echovirus. Virus studies in sludge-amended soils have dealt mainly with the transport and survival patterns of enteroviruses and more work is needed on the behavior of other enteric viruses, namely rotaviruses, in soils receiving wastewater sludges.

TABLE 5-8. Survival of poliovirus type 1 and echovirus type 1 following suspension in liquid sludge and subsequent application to large soil cores of Eustis fine sand exposed to natural conditions (11 October 1978-1 November 1978)

Sampling date (1978)	Days after the beginning of experiment	Cumulative rainfall[a] (cm)	Sludge[b] solids content (%, wt/wt)	Soil moisture (%, wt/wt)	No. of viruses (PFU/g dry weight of sludge or soil)			
					Echovirus		Poliovirus	
					LC1	LC2	LC3	LC4
LIQUID SLUDGE SAMPLE								
11 Oct.	0	0	7.0[c]	--[d]	8.6×10^4	8.6×10^4	2.6×10^7	2.6×10^7
DRYING SLUDGE SAMPLE								
14 Oct.	3	0	38.0	--	1.6×10^4	1.3×10^4	1.3×10^6	2.9×10^6
Sludge mixed with the top 2.5 cm of soil on day 3								
SOIL SAMPLES (top 2.5 cm)								
14 Oct.	3	0	--	7.5	2.8×10^2	1.9×10^2	4.3×10^4	1.9×10^4
16 Oct.	5	0	--	3.0	6.9×10^1	1.6×10^1	3.5×10^3	2.1×10^2

19 Oct.	8	0	--	1.0	5.6×10^1	6.3×10^1	3.6×10^3	2.1×10^2
01 Nov.	21	0.13	--	1.0	0	0	0	0

[a]Rainfall data were measured at the experimental site as described on page 196.

[b]One inch or 2.5 cm (254 m^3/ha) of lagooned sludge (see Table 5-3 for sludge characteristics) seeded with a total of 2.9×10^6 PFU (or 8.6×10^4 PFU/g dry weight of sludge) or a total of 8.6×10^8 PFU (or 2.6×10^7 PFU/g dry weight of sludge) of echovirus or poliovirus, respectively (see Table 5-3), was applied to large soil cores of Eustis fine sand (LC1 through LC4--see Figure 5-1). The large soil cores were 33 cm in length and 15.5 cm internal diameter; they consisted of the Ap and A21 horizons of the Eustis fine sand (see Table 4-3). The seeded sludge was allowed to soak in and dry on top of the soil for 3 days before being mixed with the top 2.5 cm of soil. The drying sludge solids and the soil were monitored for the presence of seeded viruses as detailed on pages 192 to 194. In addition to the large soil cores, virus-seeded sludge was also similarly applied to small soil cores (33 cm in length and 5.0 cm internal diameter) of Eustis fine sand for the purpose of evaluating viral transport only. Leachates from all soil cores were monitored for viruses as shown in Table 5-9.

[c]In the case of the liquid sludge, sludge solids content was expressed as percentage on a weight-to-volume basis.

[d]A dash means not done or not applicable.

TABLE 5-9. Analysis for the presence of poliovirus type 1 (Sabin) and echovirus type 1 (Farouk) in soil leachates collected after natural rainfall from soil cores which had been treated with 2.5 cm of seeded liquid sludge (11 October 1979-20 January 1979)

Dates of leachates collected	Cumulative rainfall[a] (cm)	Soil core[b] number[c]	Cumulative leachate volume (ml)	Cumulative number of pore volumes[d]	Cumulative virus breakthrough (total PFU)	Cumulative percent of total virus applied	Range of conductivity values of leachates collected (μmho/cm)	Range of pH values of leachates collected
01 Dec. 78-20 Jan. 79	24.95	SC1	400 (20.4)[e]	1.7	0	0	140-625	5.8-7.0
01 Dec. 78-20 Jan. 79	24.95	SC2	385 (19.6)	1.6	0	0	135-710	6.3-7.0
01 Dec. 78-20 Jan. 79	24.95	C1	750 (4.0)	0.3	0	0	375-800	6.3-6.8
01 Dec. 78-20 Jan. 79	24.95	C2	980 (5.2)	0.5	0	0	190-975	6.1-6.3
01 Dec. 78-20 Jan. 79	24.95	C3	920 (4.9)	0.4	0	0	280-1200	5.9-6.9
28 Dec. 78-20 Jan. 79	24.95	C4	410 (2.2)	0.2	0	0	560-875	6.0-6.9

[a]The cumulative rainfall (cm) values represent the total rainfall from the beginning of the experiment on 11 October 1978.

[b]One inch (2.5 cm) of lagooned sludges seeded with a total of 8.6×10^8 (9.3×10^7 for the small core) PFU of poliovirus or 2.9×10^6 (3.1×10^5 for the small core) PFU of echovirus was applied on top of soil cores. The soil cores were exposed to natural conditions.

[c]Echovirus was seeded in the sludge applied to small core 1, core 1, and core 2. Poliovirus was seeded in the sludge applied to small core 2, core 3, and core 4.

[d]One pore volume for the large soil cores and the small soil cores equals 2178 ml and 234 ml, respectively.

[e]Values in parentheses represent the number of centimeters of cumulative leachate volume.

The results presented above show that the movement of viruses in sludge-amended soil cores exposed to natural conditions is limited due to the following factors:

1. Viruses were retained with the sludge solids on top of the soil profile.
2. Viruses were inactivated in the natural environment (laboratory leaching studies involving viruses are usually completed in one day and neglect to take viral inactivation into account - see Figure 4-9).
3. Rain water moved through the soil cores under natural, unsaturated flow conditions.

Several investigators have demonstrated that,under natural conditions, rain water flows rapidly down macropores causing only partial displacement of the initial soil water and contributing greatly to groundwater recharge (Elrick and French 1966; McMahon and Thomas 1974; Nielsen and Biggar 1961; Quisenberry and Phillips 1976, 1978; Thomas and Phillips 1979; also see review by Burge and Parr 1980). Under these conditions, it can be hypothesized that viral particles associated with the immobile, matrix, soil water (i.e., in micropores) in the sludge-amended cores were probably bypassed by the fast moving rain water. In laboratory studies involving saturated flow conditions, the reverse would probably occur; that is, the viral particles would move rapidly with the soil water in the macropores and this was demonstrated in Figure 4-9.

CHAPTER VI
MONITORING OF INDIGENOUS ENTEROVIRUSES
AT TWO SLUDGE DISPOSAL SITES IN FLORIDA

Introduction

Wastewater effluents and sludges are being disposed of on land with increasing frequency (U.S. Environmental Protection Agency 1974, 1977, 1978b). It has been suggested, however, that this practice may result in the dissemination of viral pathogens throughout the disposal site (e.g., soil and groundwater) and adjacent areas (e.g., surface waters) (Burge and Marsh 1978; Elliott and Ellis 1977; Foster and Engelbrecht 1973; Kowal and Pahren 1978; Pahren 1980; Pahren et al. 1979). Consequently, numerous studies have been conducted to determine the fate of indigenous enteric viruses following wastewater effluent application to land at several locations in the United States (Dugan et al. 1975; England et al. 1965; Gilbert et al. 1976a, 1976b; Merrell and Ward 1968; Schaub and Sorber 1977; Vaughn et al. 1978; Wellings et al. 1974, 1975, 1978). In contrast, viral persistence and transport at sludge disposal sites has not been adequately investigated (see review by Bitton et al. 1979b). Hurst et al. (1978) found that indigenous enteroviruses (mostly echovirus type 7) were inactivated at the rate of 2 $\log_{10}$ per week in aerobically digested-dewatered sludge undergoing further drying in piles on land (temperature range: 20 to 31°C). These investigators demonstrated that viral inactivation in the sludge piles was directly related to the loss of moisture. Viruses

could not be detected in the sludge after 3 months of drying on the soil surface (see Hurst et al. 1978). When sludge was injected below the soil surface, viral persistence was found to be prolonged. At a sludge injection site in Butte, Montana, for example, Moore et al. (1978) recovered indigenous enteric viruses (1.1 PFU per gram dry wt. of soil obtained from the sludge injection depth--approximately 15 cm) from soil sampled 6 months (mostly fall and winter seasons; thus, low temperatures were encountered) after sludge injection had been discontinued. Viral inactivation was significantly accelerated in injected sludge which had seeped to the soil surface at the Butte site and had been subjected to air drying (see Moore et al. 1978). Indigenous enteric viruses at sludge disposal sites have been shown not to be transported to surface waters (Zenz et al. 1976) or groundwater (Farrah et al. 1981a).

In this chapter, results of viral monitoring at two sludge disposal sites in Florida are presented. The City of Gainesville (Florida) sludge disposal site adjacent to Lake Kanapaha was monitored (monitored the sludge applied to the site, topsoil and groundwater) for indigenous enteroviruses on a monthly basis from December 1977 through February 1978. Topsoil from the sludge disposal site at the West Florida Agricultural Experiment Station, Jay, Florida, was also monitored for indigenous enteroviruses on a monthly basis from June 1978 through January 1979. The applied sludge and groundwater at the Jay site were monitored for indigenous enteroviruses by Farrah et al. (1981a) and their results are summarized herein. The information gained from viral monitoring at these two sludge disposal sites should be of

value in the ultimate assessment of the actual viral risk of sludge application to soils.

Materials and Methods

Sludge Disposal Sites

Two sludge disposal sites were monitored for indigenous enteroviruses and they are described below.

Kanapaha site. The City of Gainesville (Florida) sludge disposal site (10 acres or 4.05 ha) adjacent to Lake Kanapaha has been in operation since August 1977. At the time of this study, the sludge applied to this site originated at the Main Street wastewater treatment plant, Gainesville. At this treatment plant, wasted sludge undergoes 180 days of aerobic digestion. The digested sludge (GDA180--see Table 3-2) is conditioned with a cationic polymer and then dewatered by centrifugation (U.S. Environmental Protection Agency 1974, 1978a; also see Figure 2-1). The conditioned-dewatered sludge was transported by tank truck to the Kanapaha site for ultimate disposal. The schematic of sludge treatment and final disposal at the Kanapaha site is shown in Figure 6-1. The conditioned-dewatered sludge was spread out onto the soil and immediately disced into the soil except when a cover crop was present. In the presence of a cover crop, the sludge was applied as a top dressing on the crop. Coastal bermudagrass was utilized during the summer months while ryegrass was used in the winter months (Gainesville-Alachua County Regional Utilities 1976).

The Kanapaha site is depicted in Figure 6-2. As shown, a 60-ft (ca. 18-m) deep well in the center (west) of the site was monitored for

FIGURE 6-1. Scheme for sludge disposal at the Kanapaha site, Gainesville, Florida

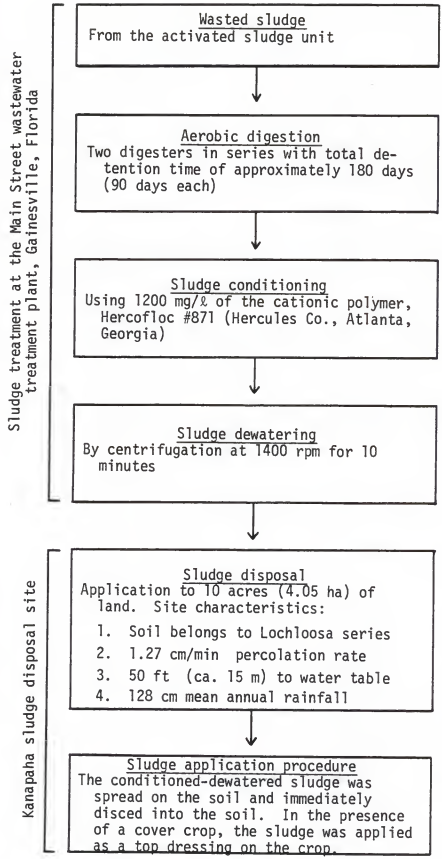
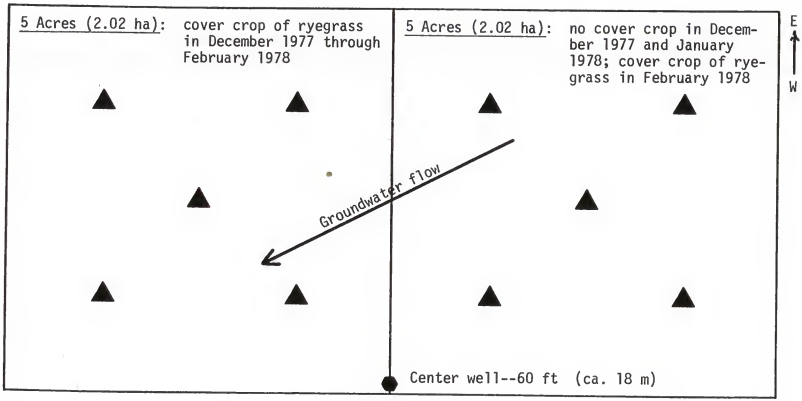

Sludge treatment at the Main Street wastewater treatment plant, Gainesville, Florida
Wasted sludge
From the activated sludge unit
Aerobic digestion
Two digesters in series with total detention time of approximately 180 days (90 days each)
Sludge conditioning
Using 1200 mg/ℓ of the cationic polymer, Hercofloc #871 (Hercules Co., Atlanta, Georgia)
Sludge dewatering
By centrifugation at 1400 rpm for 10 minutes
Kanapaha sludge disposal site
Sludge disposal
Application to 10 acres (4.05 ha) of land. Site characteristics:
1. Soil belongs to Lochloosa series
2. 1.27 cm/min percolation rate
3. 50 ft (ca. 15 m) to water table
4. 128 cm mean annual rainfall
Sludge application procedure
The conditioned-dewatered sludge was spread on the soil and immediately disced into the soil. In the presence of a cover crop, the sludge was applied as a top dressing on the crop.

FIGURE 6-2. Diagram of the Kanapaha sludge disposal site, Gainesville, Florida

Conditioned-dewatered sludge from the Main Street wastewater treatment plant (Gainesville) was applied to the 10 acres (4.05 ha) of land as shown in Figure 6-1. From August 1977 to February 1978 (i.e., last month of sampling in this study), a total of 3.7 inches (9.4 cm) of sludge were applied to the site. The soil found at this site belongs to the Lochloosa series. A 200-g composite topsoil sample [40 grams per sampling point (sampling points designated by triangles)] was obtained from each 5 acre plot and analyzed for the presence of indigenous enteroviruses. Groundwater from the center well [60 ft (ca. 18 m)] in the disposal site was also monitored for indigenous enteroviruses.


5 Acres (2.02 ha): cover crop of ryegrass in December 1977 through February 1978
5 Acres (2.02 ha): no cover crop in December 1977 and January 1978; cover crop of ryegrass in February 1978
E
W
Groundwater flow
Center well--60 ft (ca. 18 m)

indigenous enteroviruses. The water table at the site has been found to be 50 ft (ca. 15 m) below the soil surface (Gainesville-Alachua County Regional Utilities 1976). Other characteristics of the Kanapaha site are shown in Figure 6-1. The groundwater flows in a northwesterly direction as shown in Figure 6-2. The topsoil at the Kanapaha site was also monitored for indigenous enteroviruses (see Figure 6-2). The soil found at the site belongs to the Lochloosa series (Gainesville-Alachua County Regional Utilities 1976). This soil series is classified as an Aquic Arenic Paleudult, loamy, siliceous, hyperthermic (Calhoun et al. 1974). Some characteristics of the typifying pedon, Lochloosa fine sand, are shown in Table 6-1.

The conditioned-dewatered sludge, topsoil, and groundwater from this site were monitored for indigenous enteroviruses on a monthly basis from December 1977 through February 1978. Wasted sludge and aerobically digested sludge (digested 90 days; GDA90 in Table 3-2) from the Main Street wastewater treatment plant (Gainesville, Florida) were also tested for the presence of indigenous enteroviruses in December 1977 and February 1978.

Jay site. Aerobically digested (1/3) and anaerobically digested (2/3) sludge from the Montclair and Main Street wastewater treatment plants of Pensacola, Florida, respectively, were transported by tank truck and discharged into a sludge lagoon located at the West Florida Agricultural Experiment Station, Jay, Florida (PDA and PDAN, respectively; see Table 3-2). Characteristics of the sludge lagoon and the scheme for sludge disposal at the Jay site are shown in Figure 6-3.

TABLE 6-1. Some characteristics of the Lochloosa soil series found at the Kanapaha site

Soil[a] horizon	Depth (cm)	Mechanical composition (%)			pH (in 1:1 water)
		Sand (2-0.05 mm)	Silt (0.05-0.002 mm)	Clay (<0.002 mm)	
Ap	0-18	88.9	7.8	3.3	4.9
A21	18-43	91.2	4.0	4.8	5.1
A22	43-71	89.2	4.5	6.3	5.2
B1t	71-81	79.3	4.7	16.0	5.1
B21tg	81-89	67.4	4.7	27.9	4.8
B22tg	89-145	66.8	3.6	29.6	4.6
B3g	145-175	59.2	2.6	38.2	4.8

[a]Typifying pedon is Lochloosa fine sand. Data were adapted from Calhoun et al. (1974).

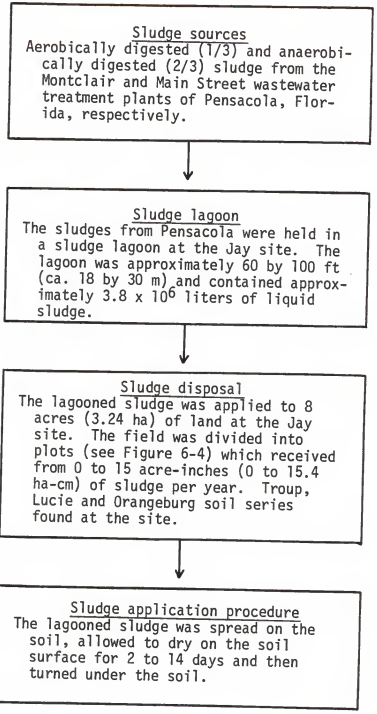


FIGURE 6-3. Scheme for sludge disposal at the West Florida Agricultural Experiment Station, Jay, Florida

Lagooned sludge (LAG--see Table 3-2) was subsequently spread on 8 acres (3.24 ha) of land at the Jay site. A diagram of the field used for sludge disposal at Jay is shown in Figure 6-4. The field site was divided into 72 plots of 40 by 120 ft (ca. 12 by 36 m) which received from 0 to 15 acre-inches (0 to 15.4 ha-cm) of sludge per year (see Figure 6-4). The applied sludge was allowed to dry on the soil surface for 2 to 14 days and was then turned under the soil (see Figure 6-3).

The soil series found at the site are Troup, Lucie, and Orangeburg (see Figure 6-4). The Troup series has been classified as a Grossarenic Paleudult, loamy, siliceous, thermic, while the Orangeburg series was classified as a Typic Paleudult, fine-loamy, siliceous, thermic (Calhoun et al. 1974). Some characteristics of the typifying pedons, Troup loamy sand and Orangeburg sandy loam, are shown in Table 6-2. No information was available on the Lucie soil series. The topsoil from plots numbered 1, 32, and 61 which received 15 acre-inches (15.4 ha-cm) of sludge per year was monitored for indigenous enteroviruses on a monthly basis from June 1978 through January 1979. In addition, the topsoil from the plot numbered 42 which received no sludge was monitored for viruses as a control. Indigenous enteroviruses in the Pensacola sludges (PDA and PDAN--see Table 3-2) added to the sludge lagoon and in the lagooned sludge (LAG--see Table 3-2) were monitored by Farrah et al. (1981a). Also, groundwater from wells on the Jay site (see Figure 6-4) was analyzed for the presence of indigenous enteroviruses by Farrah et al. (1981a).

FIGURE 6-4. Diagram of the sludge disposal site at the West Florida Agricultural Experiment Station, Jay, Florida

Lagooned sludge (LAG--see Table 3-2) has been applied, for some years, to 8 acres (345,600 ft^2 or 3.24 ha) of land at the West Florida Agricultural Experiment Station. As depicted in the figure, the disposal site is divided into 72 plots (plot number is shown on the top of each plot) of 40 by 120 ft (ca. 12 by 36 m) which received from 0 to 15 acre-inches (0 to 15.4 ha-cm) of sludge per year (acre-inches applied per year is shown in the bottom of each plot). The soil series found at the disposal site are Troup, Lucie, and Orangeburg. The topsoils in plots numbered 1, 32, 42, and 61 (designated by triangles) were monitored for the presence of indigenous enteroviruses. Groundwater from 3 wells in the disposal site (designated by hexagons) and one well near the sludge lagoon (not shown) also was monitored for enteroviruses by Farrah et al. (1981a). The water table at these well sites was 40 to 60 ft (ca. 12 to 18 m) below the surface.

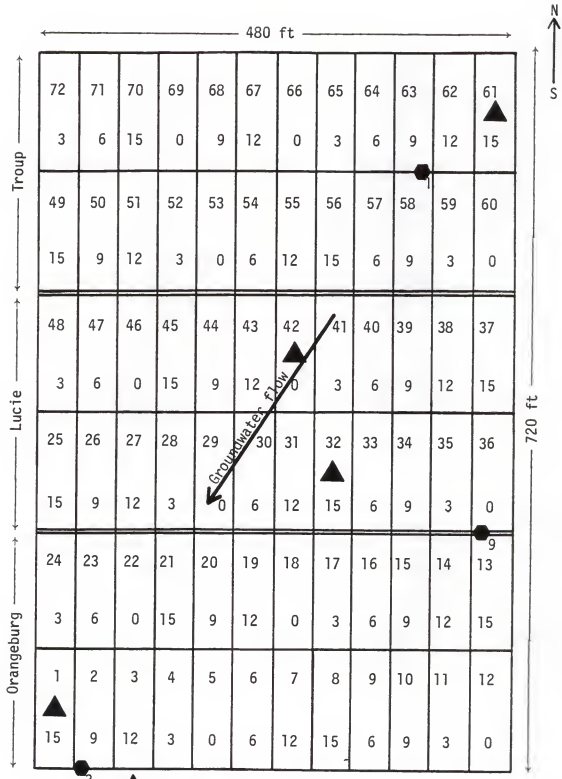

480 ft
720 ft
N
S
Troup
Lucie
Orangeburg
Groundwater Flow
72 3
71 6
70 15
69 0
68 9
67 12
66 0
65 3
64 6
63 9
62 12
61 15
49 15
50 9
51 12
52 3
53 0
54 6
55 12
56 15
57 6
58 9
59 3
60 0
48 3
47 6
46 0
45 15
44 9
43 12
42 0
41 3
40 6
39 9
38 12
37 15
25 15
26 9
27 12
28 3
29 0
30 6
31 12
32 15
33 6
34 9
35 3
36 0
24 3
23 6
22 0
21 15
20 9
19 12
18 0
17 3
16 6
15 9
14 12
13 15
1 15
2 9
3 12
4 3
5 0
6 6
7 12
8 15
9 6
10 9
11 3
12 0
Soils monitored in this study
Wells (40-60 ft) monitored by Farrah et al. (1981)

TABLE 6-2. Some characteristics of the Troup and Orangeburg soil series found at the Jay site

Soil[a]	Soil horizon	Depth (cm)	Mechanical composition (%)			pH (in 1:1 water)
			Sand (2-0.05 mm)	Silt (0.05-0.002 mm)	Clay (<0.002 mm)	
Troup loamy sand	Ap	0-15	79.0	13.7	7.3	6.1
	A21	15-41	79.0	13.2	7.8	5.8
	A22	41-71	80.0	12.2	7.8	5.4
	A23	71-91	80.5	12.5	7.0	5.4
	A24	91-117	80.3	11.9	7.8	5.4
	B1t	117-132	76.0	9.6	14.4	5.4
	B21t	132-147	62.0	10.0	28.0	5.2
Orangeburg sandy loam	Ap	0-20	77.5	13.7	8.8	4.9
	B1t	20-36	69.6	13.6	16.8	6.1
	B21t	36-64	63.5	12.6	23.9	6.3
	B22t	64-119	56.9	8.6	34.5	6.3
	B23t	119-185	61.8	8.1	30.1	5.6

[a]Data were adapted from Calhoun et al. (1974).

Virus Recovery Procedures

Sludge. Samples (1 to 4 liters) of wasted sludge, aerobically digested sludge (90 days) and dewatered-conditioned sludge from the Main Street wastewater treatment plant (Gainesville) were collected in sterile Nalgene bottles and transported to the laboratory. The pH and solids content of each sludge sample was determined as described in Chapter III (see pages 56-59).The sludge samples (i.e., total volume) were then centrifuged at 1400 x g for 10 minutes at 4°C. The sludge supernatants were discarded. The sludge solids-associated viruses were eluted and further concentrated using a modification of the glycine method developed by Hurst et al. (1978). This method is detailed in Chapter III (see pages 65 to 67).The filter and pellet concentrates produced were then assayed for viruses as described below.

Soil. Composite topsoil samples (200 wet grams) from the Kanapaha site were obtained as shown in Figure 6-2. Indigenous enteroviruses were recovered from these soil samples using the procedure described by Hurst and Gerba (1979). This method consisted of mixing each 200-g sample of wet soil with 600 ml of 0.25 M glycine buffer, 0.05 M ethylenediaminetetraacetic acid (EDTA), pH 11.5. If necessary, the pH of the mixture was adjusted to between 11.0 and 11.5 by the addition of 1 M glycine buffer, pH 11.5. The samples were vigorously shaken by hand for 30 seconds and then shaken on a rotating shaker for 4.5 minutes. The samples were subsequently centrifuged at 1400 x g for 4 minutes at 4°C (all centrifugation was performed using a Sorvall RC5-B centrifuge, Ivan Sorvall Inc., Norwalk, Connecticut). The

supernatants (i.e., the soil eluates) were recovered and immediately adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0. The entire procedure described above was performed in approximately 10 minutes. Viruses in the soil eluates were concentrated by organic flocculation (Katzenelson et al. 1976b) as follows. The eluates were adjusted to pH 3.5 by the addition of 1 M glycine buffer, pH 2.0, and to 0.06 M aluminum chloride. The flocs produced were pelleted by centrifugation at 8000 x g for 10 minutes at 4°C. The supernatants and pellets produced were treated separately. The supernatants were passed through a series of 3.0-, 0.45- and 0.45-µm Filterite filters (Filterite Corp., Timonium, Maryland) in a 47-mm holder. Adsorbed viruses were eluted from the filters with 7 ml of phosphate-buffered saline (PBS) containing 10% fetal calf serum (FCS), pH 9.0. The filter eluates were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0, and assayed for viruses as described below. The pellets previously obtained by centrifuging the samples at pH 3.5 (and at 0.06 M aluminum chloride) were mixed with five volumes of PBS containing 10% FCS, pH 9.0. The mixtures were adjusted to pH 9.0 by the addition of 1 M glycine buffer, pH 11.5, vortexed for 30 seconds and then centrifuged at 8000 x g for 10 minutes at 4°C. The supernatants were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0. Viruses in these samples were further concentrated by hydroextraction at 4°C using polyethylene glycol (i.e., Carbowax PEG 20,000 from Fisher Scientific Co., Pittsburgh, Pennsylvania). The hydroextracted samples were dialyzed against PBS at 4°C in order to remove

any possible cytotoxicity and were then assayed for viruses as described below.

Composite topsoil samples (100 wet grams) were obtained from the plots shown in Figure 6-4 at the Jay site. Indigenous enteroviruses were recovered from these soil samples using the procedure described by Bitton et al. (1979a). This method consisted of mixing each 100-g sample of wet soil with 200 ml of 0.5% (wt./vol.) isoelectric casein (Difco Laboratories, Detroit, Michigan), pH 9.0. If necessary, the pH of the mixture was adjusted to between 9.0 and 9.2 by the addition of 5 M Trizma base (Sigma Chemical Co., St. Louis, Missouri). The samples were vigorously shaken by hand for 30 seconds and then shaken on a rotating shaker for 15 minutes. The samples were subsequently centrifuged at 1400 x g for 4 minutes at 4°C. The supernatants (i.e., the soil eluates) were recovered and immediately adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0. Viruses in the soil eluates were concentrated by organic flocculation (Katzenelson et al. 1976b) as follows. The eluates were adjusted to pH 4.4 by the addition of 1 M glycine buffer, pH 2.0. The flocs produced were pelleted by centrifugation at 160 x g for 1 minute at 4°C. The supernatants were discarded. The pellets were mixed with 2 ml of 0.15 M Na_2HPO_4, pH 9.0. The mixtures were adjusted to neutral pH by the addition of 1 M glycine buffer, pH 11.5, and then magnetically stirred until the pellets were completely resolubilized. The samples were subsequently centrifuged at 14,000 x g for 10 minutes at 4°C. The supernatants were adjusted to neutral pH (i.e., if necessary) and FCS was added to a final concentration of 2%. Viruses in these samples were further concentrated by

ultracentrifugation at 120,000 x g for 2 hours at 5°C in a Ti-60 rotor using a Beckman model L3-50 ultracentrifuge (Beckman Instruments, Fullerton, California). The pellets produced were suspended in 1 ml of FCS. The concentrated samples were sterilized by passage through 0.25-µm Filterite filters in 13-mm holders and were then assayed for viruses as described below.

Groundwater. Groundwater from a 60-ft (ca. 18-m) well on the Kanapaha site (see Figure 6-2) was monitored for indigenous enteroviruses. Each groundwater sample of 100 gallons (ca. 384 liters) was hand pumped into a 100-gallon tank and was concentrated by membrane filtration (Farrah et al. 1976; Hill et al. 1971; Shuval and Katzenelson 1972; Sobsey et al. 1973; Sobsey et al. 1980b) in the field (see Figure 6-5) as follows. The water was adjusted to pH 3.5 by the addition of 0.2 N HCl and adjusted to 0.0005 M aluminum chloride. The treated water was then passed through a 10-in (ca. 25-cm), 0.25-µm pore size Filterite filter. The filter was then treated with 800 ml of 0.05 M glycine buffer, pH 11.5. The glycine solution was permitted to remain in contact with the filter for 1 minute, was removed and then was adjusted to neutral pH by the addition of 1 M glycine buffer, pH 2.0. The neutralized sample was transported to the laboratory, and within 1 hour, it was adjusted to pH 3.5 by the addition of 1 M glycine buffer, pH 2.0, and passed (without prior centrifugation) through a series of 3.0- and 0.45-µm Filterite filters in a 47-mm holder. Adsorbed viruses were eluted from the filters with 7 ml of PBS containing 10% FCS, pH 9.0. The filter eluate was adjusted to neutral pH by

FIGURE 6-5. Concentration of groundwater by membrane filtration for the detection of indigenous enteroviruses at the Kanapaha site

Groundwater from a 60-ft (ca. 18-m) well is shown being hand pumped into a small bucket (A). Once 100 gallons (ca. 384 liters) of water are collected in the large tank shown (B), the water is treated as described in the Materials and Methods section and then passed through a 10-in (ca. 25-cm), 0.25-µm pore size Filterite filter (placed in the filter housing C as shown). The filter is subsequently treated for virus recovery as described in the Materials and Methods section.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A
B
C

the addition of 1 M glycine buffer, pH 2.0, and assayed for viruses as described below.

Viral Assays

Samples were serially diluted, if necessary, in PBS containing 2% FCS (see Appendix for more details on the composition of this solution) and then assayed for indigenous enteroviruses on BGM cell cultures prepared as described in Chapter III (see pages 53 and 55). Inoculated cell cultures were examined for cytopathic effects for up to three weeks. Cell cultures showing cytopathic effects were passed and viral isolates were titered according to the procedures of Farrah et al. (1981a). The 50% tissue culture infective dose ($TCID_{50}$) was determined for samples containing indigenous enteroviruses.

Weather Data

Kanapaha site. Weather data were not available for this sludge disposal site.

Jay site. Weather data were collected at the West Florida Agricultural Experiment Station, Jay, Florida, and kindly provided by the station's staff. Mean monthly air temperature (maximum and minimum) and total monthly precipitation from September 1977 through March 1979 are reported in Figure 6-6.

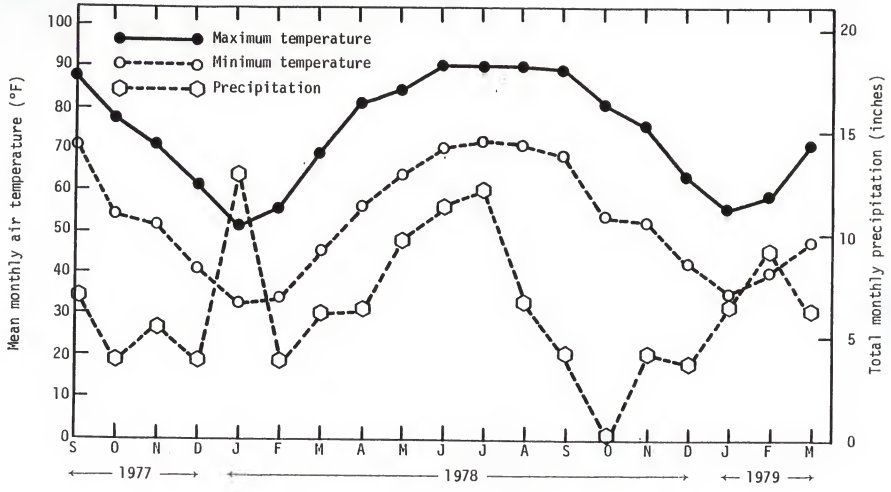

FIGURE 6-6. Weather data for the West Florida Agricultural Experiment Station, Jay, Florida

Data were collected at the station and can be converted to metric units with the following: °C = 5/9 (°F - 32) and 1 in = 2.54 cm.

Results and Discussion

Kanapaha Sludge Disposal Site

Although indigenous enteroviruses were readily recovered from the wasted sludge solids of the Main Street wastewater treatment plant (Gainesville, Florida), aerobic digestion for 90 days reduced the solids-associated viruses in sludge to almost undetectable levels (see Table 6-3). At this treatment plant, the sludge was further digested aerobically for an additional 90 days (i.e., total digestion time of 180 days), conditioned with a cationic polymer, dewatered by centrifugation, and then disposed of at the Kanapaha site. As shown in Table 6-3, indigenous enteroviruses could not be recovered from the conditioned-dewatered sludge solids. In light of this fact, it is understandable that no indigenous enteroviruses were ever detected in topsoil and groundwater samples from the Kanapaha site (see Table 6-4). Thus, apparently by increasing the sludge digestion time at the wastewater treatment plant, the viral hazard of sludge disposal on land was eliminated.

Jay Sludge Disposal Site

Lagooned sludge (2/3 anaerobic and 1/3 aerobic) has been applied to land for many years at the Jay site. The sludge is allowed to dry on top of the soil for 2 to 14 days, and then is turned under the soil. As shown in Table 6-5, indigenous enteroviruses were not detected in 100-g topsoil samples obtained, over an eight-month period, from plots at the Jay site which received 15 acre-inches of lagooned sludge yearly. Farrah et al. (1981a) readily recovered

TABLE 6-3. Indigenous enteroviruses associated with sludges from the Main Street wastewater treatment plant, Gainesville, Florida

Sampling date	Sludge[a] type	Viruses detected ($TCID_{50}$[b]/g dry wt.)
December 1977 and February 1978	Wasted	11 to 25
December 1977 and February 1978	Aerobically digested[c] (90 days)	0.3 to 1.2
December 1977, January 1978, and February 1978	Conditioned-dewatered[d]	0

[a]Sludge samples of 1 to 4 liters were centrifuged at 1400 x g for 10 min at 4°C. The sludge supernatants produced were discarded. The sludge solids were tested for the presence of viruses.

[b]Refers to the 50% tissue culture infective dose.

[c]Identified as sludge GDA90 in Table 3-2.

[d]Sludge aerobically digested for 180 days (GDA180--see Table 3-2) is conditioned with a cationic polymer and then dewatered by centrifugation at the Main Street plant. The conditioned-dewatered sludge was applied to the Kanapaha site (see Table 6-4 for data on the viral monitoring of the Kanapaha site).

TABLE 6-4. Analysis of topsoil and groundwater samples from the Kanapaha site for the presence of indigenous enteroviruses

Sampling date	Cumulative amount of sludge[a] applied by the sampling date [in (cm)]	Sample	Viruses detected
December 1977	2.6 (6.5)	Topsoil[b]	0
		Groundwater[c]	0
January 1978	2.6 (6.5)	Topsoil[b]	0
		Groundwater[c]	0
February 1978	3.7 (9.3)	Topsoil[b]	0
		Groundwater[c]	0

[a]Application of conditioned-dewatered sludge (see Table 6-3) to the Kanapaha site began in August 1977.

[b]Composite topsoil samples (200 wet grams) were obtained monthly from the Kanapaha site as shown in Figure 6-2 and were tested for the presence of viruses.

[c]Groundwater (100 gallons or 384 liters) from a 60-ft (ca. 18-m) well on the Kanapaha site (see Figure 6-2) was monitored monthly for the presence of viruses.

TABLE 6-5. Analysis of topsoil samples from the Jay site for the presence of indigenous enteroviruses

Soil plot no.[a]	Amount of lagooned sludge[b] applied yearly [acre-in (ha-cm)]	Total number of grams of topsoil[c] sampled	Viruses detected
1	15 (15.4)	800	0
32	15 (15.4)	800	0
42	0	800	0
61	15 (15.4)	800	0

[a]The locations of these soil plots at the Jay site are shown in Figure 6-4.

[b]The viral content of lagooned sludge is shown in Table 6-6.

[c]A composite topsoil sample of 100 wet grams was taken from each soil plot monthly from June 1978 through January 1979 and was tested for the presence of viruses.

indigenous enteroviruses from the digested sludges added to the lagoon, and also from the lagooned sludge (see Table 6-6). However, these investigators observed a rapid decline in the number of enteroviruses associated with lagooned sludge which had been applied to land. In fact, indigenous enteroviruses were almost undetectable in lagooned sludge allowed to dry for only two days on the soil surface at the Jay site (60% sludge solids content--see Table 6-6). It follows that enteroviruses are not likely to be detected in topsoil samples. Thus, allowing the sludge to dry on top of the soil before being mixed with the soil results in the inactivation of all or most of the viruses present (this was also demonstrated using seeded viruses in Chapter V). This may be an advantage over sludge injection into soils (Moore et al. 1978), where viruses can survive for longer periods of time. Despite the numerous advantages of sludge injection (aesthetic acceptability, and minimal odor and runoff), surface spreading of sludge may result in the inactivation of viruses at accelerated rates. It should be pointed out that Farrah et al. (1981a) were unable to detect any viruses in groundwater samples obtained from the Jay site (see Table 6-6). It appears that at this sludge disposal site, as was the case at the Kanapaha site described above, enteroviruses pose a minimal hazard with respect to soil and groundwater contamination.

TABLE 6-6. Analysis of sludge and groundwater samples from the Jay site for the presence of indigenous enteroviruses

Sample[a]	Viruses detected ($TCID_{50}$[b]/g dry wt.)	Viruses identified
Digested sludge[c] added to the sludge lagoon	2 to 260	Poliovirus 1, 2, and 3, echovirus 1 and 7, and coxsackievirus B4
Aerobic	14 to 260	
Anaerobic	2 to 7	
Lagooned sludge[d] (3%)[e]	< 0.1 to 100	Poliovirus 1 and 2, echovirus 7 and 15, and coxsackievirus B4
Lagooned sludge applied to land	<0.01 to 4.6	Poliovirus 1, echovirus 1, 4, and 7, and coxsackievirus B4
Day 0 (9%)[e]	1.4 to 4.6	
Day 2 (60%)[e]	0.10 to 0.72	
Day 9 (81%)[e]	<0.01 to 0.02	
Groundwater[f]	0	None

[a]Data were adapted from Farrah et al. (1981a).

[b]Refers to the 50% tissue culture infective dose.

[c]Aerobically digested and anaerobically digested sludge from the Montclair and Main Street wastewater treatment plants of Pensacola, Florida, and Pensacola, Florida, respectively (PDA and PDAN, respectively; see Table 3-2). Digested sludge samples were obtained from 17 February 1978 to 12 February 1979.

[d]Lagooned sludge samples were obtained on a monthly basis from 17 February 1978 to 24 January 1979.

[e]Sludge solids content was expressed as a percentage on a weight to volume basis.

[f]Groundwater from several wells at the Jay site (see Figure 6-4) was monitored for the presence of viruses. Over a 1-year period, a total of 5,950 liters (1,100 to 2,650 liters per well) of groundwater was tested.

CHAPTER VII
EFFECT OF HYDROSTATIC PRESSURE ON THE
SURVIVAL OF POLIOVIRUS SEEDED IN GROUNDWATER AND SEAWATER

Introduction

At several disposal sites in the United States receiving wastewater effluent, indigenous enteroviruses have been recovered from groundwater (Dugan et al. 1975; Schaub and Sorber 1977; Vaughn et al. 1978; Wellings et al. 1974 and 1975). Although indigenous enteroviruses were not detected in the groundwater from the two sludge disposal sites described in Chapter VI (see pages 244 to 249; also see Farrah et al. 1981a), poliovirus type 2 was isolated from a 28-foot (8.5-m) deep well and a 58-foot (17.7-m) deep companion well at a sludge disposal site in St. Petersburg, Florida (Wellings et at. 1978). In addition to the application of wastes to land, poor engineering practices (e.g., wells not properly sealed and cesspools near wells) have also been demonstrated to result in the contamination of groundwater with viral and other pathogens (Allen and Geldreich 1975 ; Mack et al. 1972; Robeck 1979).

It appears, therefore, that viruses sometimes find their way into our groundwater supplies. Unfortunately, we know little about the survival of viruses in the groundwater environment. In this environment, elevated hydrostatic pressures are likely to be encountered (McNabb and Dunlap 1975). Such elevated pressures have been found to affect water chemistry (Distéche 1959; Hamann 1963; Hamann and Strauss 1955; Horne and Johnson 1966), and the survival of bacteria (Baross et al. 1975; Hedén 1964; Horvath and Elkan 1978; Jannasch et al. 1976;

Morita and ZoBell 1956; Zobell and Cobet 1962; ZoBell and Johnson 1949) and tobacco mosaic virus (Johnson et al. 1948; Lauffer and Dow 1941). In this chapter, the effect of elevated hydrostatic pressure on the survival of poliovirus seeded in groundwater was investigated. For comparative purposes, virus survival in seawater under elevated pressure was also studied.

Materials and Methods

Virus and Viral Assays

Poliovirus type 1 (strain LSc) was used in the research reported in this chapter. Some general properties of polioviruses are shown in Table 3-1. Stocks of poliovirus were prepared as described in Chapter III (see page 53). Viral stocks were kept at -70°C until used. Poliovirus was assayed by the plaque technique as described in Chapter III (see pages 53-56). Each viral count shown represents the average of triplicate counts. The numbers of viruses were expressed as plaque-forming units (PFU).

Water Samples

The seawater used in this study was sampled at the Mantanzas Inlet on Florida's east coast. The groundwater sample was obtained from a 1200-foot deep well at the Kanapaha wastewater treatment plant, Gainesville, Florida. The water samples were collected in sterile Nalgene carboys, transported to the University of Florida (Gainesville) laboratory and then immediately refrigerated. No chlorine residual was found in these water samples (i.e., by the orthotolidine test). The pH of each water sample was measured using a digital pH meter model 125 from Corning (Corning, New York). The conductivity of each water

sample was measured using a YSI model 33 S-C-T meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). The conductivity and pH of each water sample are shown in Table 7-1. The water samples were neither filtered nor autoclaved prior to use.

Poliovirus Exposure to Hydrostatic Pressures

Poliovirus was added to 75 ml of either seawater or groundwater (water sample was temperature acclimated for experimental trial) and the solution was mixed for 1 minute. The water sample was then assayed in order to determine the initial virus concentration. Following the initial viral assay, 65 ml of the virus-seeded water sample was pressurized (i.e., to between 500 and 4000 psi) directly in a pressure chamber (virus-free and temperature acclimated for experimental trial) described by Horvath and Elkan (1978). The remaining 10 ml of virus-seeded water was left at atmospheric pressure (i.e., 14.7 psi). An atmospheric pressure control sample was always run with each elevated pressure trial. The elevated and atmospheric pressure water samples were then placed at the same temperature (i.e., 2°C or 24°C) for a known period of time (2, 8 and 24 hours). At the end of the experimental trial, the pressure was released, and the water sample was transferred to sterile glassware and assayed for poliovirus. The atmospheric pressure water sample was also assayed for poliovirus. Two experimental trials were run at each elevated pressure condition tested. Poliovirus recovery after exposure to elevated pressure was expressed as a percentage of the viral titer in the atmospheric pressure sample.

TABLE 7-1. Conductivity and pH of water samples used in this study

Water sample	Conductivity (μmho/cm at 25°C)	pH
Seawater	40,000	7.7
Groundwater	475	7.9

Results and Discussion

Initially, it was important to determine the effect of elevated pressure on the temperature and pH of groundwater. As shown in Table 7-2, the pH and temperature of groundwater was not markedly changed following pressurization at 3000 psi for 24 hours.

The inactivation of poliovirus in seawater subjected to 1000 psi of hydrostatic pressure was found to increase as the pressurization time was increased from 2 to 24 hours (see Table 7-3). After 24 hours of exposure to 1000 psi of hydrostatic pressure, only 15.6% of seeded poliovirus was recovered. Clearly, poliovirus in seawater was inactivated at an accelerated rate under 1000 psi of pressure relative to the control at atmospheric pressure. No such inactivation was observed for poliovirus in groundwater even after exposure to as high as 4000 psi of hydrostatic pressure (see Table 7-4). Thus, hydrostatic pressures in groundwater are not likely to increase viral inactivation.

High hydrostatic pressures have been previously shown to inactivate bacterial enzyme systems (Morita 1967; Morita and ZoBell 1956), to retard the growth of mesophilic terrestrial bacteria (Horvath and Elkan 1978; ZoBell and Cobet 1962; ZoBell and Johnson 1949), and to accelerate the death rate of mesophilic bacteria (Baross et al. 1975; Morita 1967; ZoBell and Cobet 1962). Although tobacco mosaic virus (Lauffer and Dow 1941), and T_2 and T_4 bacteriophages (Hedén 1964) have been demonstrated to be inactivated at pressures in excess of 1900 atm, little work had previously been done on the effect of hydrostatic pressure on animal viruses. The research presented above is only preliminary and more work is needed on the effect of hydrostatic pressure on viruses.

TABLE 7-2. Effect of hydrostatic pressure on the temperature and pH of groundwater

Pressure[a] (psi)	Mean temperature (°C)	Mean pH
14.7 (atmospheric)	24	8.1
3,000	25	8.3

[a]Pressurization time was 24 hours.

TABLE 7-3. Effect of pressurization time on the survival of poliovirus type 1 seeded in seawater at 2°C

Pressurization time (hr)	Pressure (psi)[a]		Initial virus concentration (PFU/ml)	Final virus concentration (PFU/ml)		Poliovirus recovery at elevated pressure (% of control)	Mean poliovirus recovery for each pressure (% ± SE)
	Initial	Final		At atmospheric pressure (control)	At elevated pressure		
2	1,000	1,000	3.5×10^3	3.1×10^3	2.9×10^3	93.5	92.0 ± 1.5
	1,000	1,000	4.2×10^3	4.2×10^3	3.8×10^3	90.5	
8	1,000	1,000	4.5×10^3	2.7×10^3	2.3×10^3	85.2	76.0 ± 9.3
	1,000	1,000	3.4×10^3	3.0×10^3	2.0×10^3	66.7	
24	1,000	800	3.5×10^3	3.5×10^3	5.7×10^2	16.3	15.6 ± 0.7
	1,000	920	4.0×10^3	2.9×10^3	4.3×10^2	14.8	

[a]One atm per 14.7 psi.

TABLE 7-4. Effect of hydrostatic pressure on the survival of poliovirus type 1 seeded in groundwater at 24°C

Pressure[a] (psi)[b]		Initial virus concentration (PFU/ml)	Final virus concentration (PFU/ml)		Poliovirus recovery at elevated pressure (% of control)	Mean Poliovirus recovery for each pressure (% ± SE)
Initial	Final		At atmospheric pressure (control)	At elevated pressure		
500	350	1.7×10^4	1.6×10^4	1.5×10^4	93.8	90.7 ± 3.2
500	420	1.6×10^4	1.6×10^4	1.4×10^4	87.5	
1,000	820	1.9×10^4	1.0×10^4	1.0×10^4	100.0	95.5 ± 4.6
1,000	900	2.0×10^4	1.1×10^4	1.0×10^4	90.9	
2,000	1,900	1.3×10^4	1.1×10^4	8.8×10^3	80.0	85.5 ± 5.5
2,000	1,800	1.6×10^4	1.1×10^4	1.0×10^4	90.9	
3,000	2,300	1.6×10^4	1.5×10^4	1.1×10^4	73.3	82.5 ± 9.2
3,000	2,300	1.8×10^4	1.2×10^4	1.1×10^4	91.7	
4,000	2,700	2.0×10^4	1.2×10^4	1.3×10^4	108.0	100.0 ± 7.6
4,000	2,800	2.0×10^4	1.4×10^4	1.3×10^4	92.9	

[a]Pressurization time was 24 hours.

[b]One atm per 14.7 psi.

CHAPTER VIII

CONCLUSIONS

Based on the findings of this study, the following conclusions can be drawn:

1. Poliovirus type 1 (LSc) was largely associated with digested, conditioned-dewatered, chemical (alum, ferric chloride and lime) and lime-stabilized, chemical sludge (alum and ferric chloride) solids.
2. Sludge type was found to affect the degree of association between seeded poliovirus and sludge solids. The mean percent of solids-associated viruses for activated sludge mixed liquors, anaerobically digested sludges and aerobically digested sludges was 57, 70 and 95, respectively. The degree of association between poliovirus and sludge solids was significantly greater for aerobically digested sludges than for the other two sludge types.
3. A smaller fraction of echovirus type 1 (Farouk) was associated with lagooned sludge solids than was the case for poliovirus.
4. The effectiveness of the glycine method in the recovery of solids-associated viruses was found to be affected by sludge type. Significantly lower mean poliovirus recovery was found for aerobically digested sludges (15%) than for mixed liquors or anaerobically digested sludges (72% and 60%, respectively).
5. Poliovirus transport studies involving soil cores treated with virus-seeded sludge were conducted under controlled laboratory and saturated flow conditions. A Red Bay sandy loam displayed a substantially greater retention capacity for poliovirus in anaerobically digested sludge than a sandy soil (i.e., Eustis fine sand). The Red Bay sandy loam soil was shown to completely retain poliovirus following the application of conditioned-dewatered, chemical (alum, ferric chloride and lime) and lime-stabilized, chemical (alum and ferric chloride) sludge.

6. The stabilization of chemical sludges (alum and ferric chloride sludges) with lime resulted in almost complete inactivation of seeded poliovirus.
7. Undisturbed soil cores of Eustis fine sand were treated with several inches of virus-seeded (poliovirus and echovirus) sludge during a two-year period. The soil cores were exposed to natural conditions and soil temperature, soil moisture and rainfall were monitored. Both viruses were found to be rapidly inactivated in the sludge during the drying process on top of the soil cores. Monitoring of the top inch of soil revealed that both viruses were inactivated with time and were undetectable after 35 days. The inactivation of both viruses in the soil appears to be affected more by soil moisture. Soil leachates collected after natural rainfall (unsaturated flow conditions) were negative for both viruses except on one occasion (only 0.0006% of total poliovirus applied was found in leachate) when heavy rainfall occurred immediately after liquid sludge application to the soil.
8. Indigenous enteroviruses were not detected in topsoil and groundwater samples from two sludge disposal sites in Florida. It appears that, at these two sludge disposal sites, enteroviruses pose a minimal hazard with respect to soil and groundwater contamination.
9. Poliovirus in seawater was found to be inactivated when subjected to 1000 psi of hydrostatic pressure for 24 hours. No such inactivation was observed for this virus in groundwater even after exposure to as high as 4000 psi of hydrostatic pressure.

This research has allowed the determination of the persistence and possible movement of pathogenic viruses in soils treated with wastewater sludge. The information gained from this study is of value in the ultimate assessment of the potential risk of viral infection to humans associated with land disposal of sludges.

APPENDIX
COMPOSITION OF MEDIA AND SOLUTIONS
USED IN ENTEROVIRUS ASSAYS

1. Gey's Balanced Salt Solution (BSS) is the common diluent for cell cultures:

Gey's A (10x):

70 grams NaCl
3.7 grams KCl
3.01 grams $Na_2HPO_4 \cdot 12H_2O$
0.237 grams KH_2PO_4
100 ml 0.1% phenol red
10 grams glucose
900 ml glass distilled water
5 ml chloroform, as a preservative

This stock solution of Gey's A is stored at room temperature unautoclaved, and is diluted 1:10 and autoclaved when needed.

Gey's B (20x):

0.42 grams $MgCl_2 \cdot 6H_2O$
0.14 grams $MgSO_4 \cdot 7H_2O$
0.34 grams $CaCl_2$
100 ml glass distilled water

Gey's C (20x):

2.25 grams $NaHCO_3$
100 ml glass distilled water

Bubble CO_2 into Gey's C until pH is less than 7.6. Dispense and tightly cap.

Gey's B and C are autoclaved without further dilution.

To make the complete Gey's Balanced Salt Solution (BSS) add:

90 parts Gey's A (1x)
5 parts Gey's B (20x)
5 parts Gey's C (20x)

2. Hepes buffer (1 M) stock solution:

47.7 grams Hepes
190 ml Gey's A (1x)

10 ml Gey's B (20x)
16 ml 2 M NaOH (8g/100 ml)

Dispense and autoclave.

3. Streptomycin-penicillin (1000x) stock solution:

Solution I: 1.0 gram streptomycin
8 ml Gey's A (1x)

Solution II: 10^6 units of penicillin
4 ml of Solution I

Solution II contains 125 mg of streptomycin and 2.5×10^5 units of penicillin per ml which is 1000x of what is required. Therefore, it must be diluted 1:1000 in the final solution.

4. Eagle's Minimal Essential Medium (MEM) using Gey's BSS plus 10% fetal calf serum (FCS) (i.e., growth medium):

300 ml Gey's A (1x)
20 ml Gey's B (20x)
20 ml Gey's C (20x)
8 ml MEM essential amino acids (50x) (International Scientific, Gary, Illinois)
4 ml vitamins (100x) (International Sci.)
4 ml glutamine (100x) (International Sci.)
0.4 ml streptomycin-penicillin stock (1000x)
40 ml FCS (International Sci.)

5. Solutions required for the removal of cells from glass (trypsinization):

Solution I (pre-trypsin wash):

300 ml Gey's A (1x)
5 ml Gey's C (20x)

Dispense and autoclave. This solution removes all traces of serum (which contains trypsin inhibitors) as well as Ca^{+2} and Mg^{+2} ions.

Solution II [1% versene (i.e., EDTA) stock in Gey's A]:

2.0 grams ethylenediamine-tetraacetic acid (EDTA)
10 ml 2 M NaOH (8g/100 ml)
20 ml Gey's A (10x)
170 ml glass distilled water

Dispense and autoclave.

Solution III (2.5% trypsin stock):

1.0 gram trypsin (Difco Laboratories, Detroit, Michigan; 1:250)
100 ml glass distilled water

Sterilize by cold filtration. Dispense in 5 ml aliquots to screw-capped test tubes and freeze for storage.

Solution IV (standard trypsin-versene solution):

100 ml Gey's A (1x)
4 ml Gey's C (20x)
4 ml stock trypsin (2.5%)
4 ml stock versene (1% in Gey's A)

This solution is good for only one day. This solution is used to remove the cells from the 32-ounce bottles in which they have been growing prior to their distribution to plaque bottles.

6. Methyl cellulose overlay for cell cultures (1% methyl cellulose plus 5% FCS):

Solution I:

300 ml glass distilled water
6 grams methyl cellulose (1500 centipoise)

Autoclave and then allow to cool to room temperature, shaking vigorously every hour to avoid layering. Refrigerate.

Solution II (2x Eagle's MEM):

350 ml glass distilled water
120 ml Eagle's MEM (10x) with Hanks' salts (International Sci.)

50 ml Gey's C (20x)
60 ml FCS (International Sci.)
25 ml Hepes buffer (1 M) stock solution
12 ml glutamine (100x) (International Sci.)
1.2 ml streptomycin-penicillin stock (1000x)

Combine equal amounts of Solutions I and II to make the methyl cellulose overlay. To 500 ml of the methyl cellulose overlay, 0.175 ml of kanamycin stock (i.e., stock supplied as a liquid at a concentration of 1 gram per 3 ml).

7. Crystal violet:

Solution I: 20 grams crystal violet
200 ml absolute ethanol

Allow this solution to sit overnight.

Solution II: 8 grams ammonium oxalate
800 ml distilled water

Mix Solutions I and II, and dilute 1:10 with tap water. This stain is used to make the plaques on the cell monolayer visible to the naked eye. In some experiments, the cells were stained, instead, with 0.5 ml of 0.5% neutral red.

8. Eagle's MEM using Gey's BSS plus 5% calf serum and 0.03 M Hepes buffer at pH 7:

This solution was used to make some virus dilutions. The solution is made by substituting, in Solution 4 above, 20 ml of calf serum (International Sci.), 12 ml of 1 M Hepes buffer stock solution and 8 ml of Gey's A (1x) for 40 ml of fetal calf serum.

9. Phosphate-buffered saline (PBS) at pH 7.4-7.6:

8.0 grams NaCl
0.2 grams KCl
1.15 grams Na_2HPO_4

0.2 grams KH_2PO_4
1000 ml deionized water

Autoclave.

10. PBS containing 2% fetal calf serum at pH 7.4 (this solution was also used to make virus dilutions):

To 490 ml of PBS, add the following aseptically:

10 ml fetal calf serum (International Sci.)
0.5 ml phenol red stock (0.5%)
0.5 ml streptomycin-penicillin stock (1000x)

11. NaCl stock (used to bring an undiluted sample to isotonicity--i.e., 0.85 grams NaCl per 100 ml):

17.0 grams NaCl
100 ml deionized water

Autoclave. Dilute 1/20 in final sample.

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BIOGRAPHICAL SKETCH

Oscar Carlos Pancorbo was born November 27, 1953, in Mantanzas, Cuba. There, he attended parochial school until his immigration to the United States in October 1961. In May 1971, he graduated from Immaculata-La Salle High School in Miami, Florida. In August 1974, he graduated from the University of Florida with a Bachelor of Science in zoology with Honors. Following graduation, he enrolled in the Graduate School of the University of Florida and in June 1976, was awarded the degree of Master of Science in environmental engineering sciences. Since August 1981, he has been employed as an Assistant Professor in the Department of Environmental Health at East Tennessee State University, Johnson City.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Gabriel Bitton, Chairman
Professor of Environmental Engineering Sciences

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Thomas L. Crisman
Associate Professor of Environmental Engineering Sciences

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W. Ernest Bolch for Dale A. Lundgren
Dale A. Lundgren
Professor of Environmental Engineering Sciences

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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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